



TO CARRY OUT THE AMELIORATIVE EFFECT OF PEPTIC ULCER VIA PHARMACOGNOSTIC STUDIES OF PHOENIX DACTYLIFERA & PHARMACOLOGICAL SCREENING OF MUSA SAPIENTUM

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ABSTRACT

Traditional medicines of all over the world possess different virgin remedies for the treatment of symptomatology's related to many ailments. Thus, they are very important for investigation on their efficacy and phytochemical constituents. There are several edible fruits and spices proposed in traditional Iranian medicine (TIM).

Present study conducted to review these fruits and spices and found evidence for their efficacy and biological mechanisms.

In future, there are many compounds may be isolated from Phoenix dactylifera and biological activities of those isolated compounds and the toxicities, if any, also would be judged as follows by performing TLC study. On several extracts it is found that Phoenix dactylifera contains lots of compounds and the leaf has different variety of activities on human body to cur evarious type of diseases. So, several other methods are required to extract more compounds. Extensive research is required to assess the biological activities of the isolated compounds along their toxicity studies. Spectroscopic techniques may help in elucidation of their structures and quantitative structure activity relationship may be tried to synthesize the analogs from where we may get less toxic drug for future use.

PHOENIX DACTYLIFERA

Fruit and seed possess antioxidant activity. The fruit ameliorated gastric ulcers via increasing gastric mucin and reducing histamine and gastrin (a gastrointestinal hormone that regulates gastric acid secretion, releases histamine, and regulates gastric endocrine cell proliferation in the plasma

MUSA SAPIENTUM

Chloroform & Ethanol extract of *Musa sapientum* (MEMS) was evaluated for its for its ulcer healing propertys.

KEYWORDS: Phytochemical, Phoenix dactylifera, Musa sapientum , Gastrointestinal, Proliferation

1.1.INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all inter dependent. The plants are indispensable to man for his life. The three important necessities of life-food, clothing & shelter and a host of other useful products are supplied to him by the plant kingdom. Nature has provided a complete storehouse of remedies to cure ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that today we possess many effective means of ensuring health care. India has an ancient heritage of traditional medicine; it is presumed that the knowledge of Ayurveda is given by Gods of a different world.

It is accepted as the oldest written medical system that is also supposed to be more effective in certain cases than modern therapies. We need not to go in any controversy regarding its origin, as Ayurveda is an independent and self-sufficient medical system, which stood with the test of time. The origin of Ayurveda has been lost in pre historic antiquity, but their characteristic concepts appear to have been nurtured between 2500 and 500 BC in India (Mukherjee; 2001). In addition to the above recorded information there is a great wealth of knowledge concerning the medicinal, narcotic and other properties of plants that is still transmitted orally from generation to generation by tribal societies, particularly Introduction those of tropical Africa, North and South America and pacific countries (Trease; 2007).

Ulcer has long been recognized as one of the most important problems in developing countries. About 70% population in developing countries relies on traditional medicine for their primary health care needs (Antwi et al., 2009).

1.2 Etiology of Peptic ulcer

Peptic ulcer disease is a major health problem. The development of gastric ulcer occurs with acid and the breakdown of mucosal defense. Peptic ulcer occurs in part of gastrointestinal tract, which is exposed to gastric acid and pepsin, i.e., the stomach and duodenum (Tripathi; 2011). Development of peptic ulcer depends on the balance between the known aggressive factors and mucosal defense mechanism. Some of the aggressive factors are gastric acid, bile salts abnormal motility, pepsin, use of non-steroidal anti-inflammatory drugs (Goel; 2002) and infections with microorganism. Mucous secretion, gastro duodenal bicarbonates production, prostaglandin synthesis (Zohary; 1993), cellular regeneration & normal tissue micro calculation protect against ulcer formation.

A number of anti-ulcer drugs like proton pump inhibitors, anti-muscarinic agents, mucosal protective agents-carbenoxolone sodium, sucralfate and analogues are available which are shown to have side effects and limitation. (Vayalil; 2002).

Peptic ulcer is one of the major ailments affecting about 60% of the human adults and nearly 80% of child population in tropical countries (Gowrishankar; 2004). Hence, we can say that gastric ulcers are caused due to imbalance between offensive and defensive factor of gastric mucosa. The anti-ulcerogenic activity of many plant products is reported due to an increase in mucosal defensive factors rather than decrease in offensive factors (Goel; 1985).

1.3 FACTORS MODIFYING GASTRIC ACID SECRETION

The gastric juice is a mixture containing hydrochloric acid, pepsin, rennin (in children), neutral chlorides, mucous, intrinsic factor and traces of potassium, ammonium and calcium.

The gastric acid and pepsin are secreted by the main gastric glands, containing highly specialized cells, present all over the body and fundus of the stomach.

The rate and the composition of secretion of main gastric glands vary considerably, depending upon the number of acid secreting cells (the parietal cell mass), emotional factors, digestive state, hormonal status and the presence of extrinsic chemical stimuli such as caffeine and histamine.

The parietal cells are located in the walls of the midsection of the parietal glands, the secretory unit of the gastric mucosa. The parietal cell has prominent cytoplasmic tubulovesicles. In addition, these glands contain chief, mucous, endocrine and somatostatin cells. Gastric acid secretion is regulated by intricate central and peripheral mechanisms.

The central mechanism acts through the vagus nerve. In addition, acetylcholine liberated from the post ganglionic nerve fibers directly stimulates the parietal cells.

Three distinct pathways deliver chemical messengers that stimulate acid secretion.

1. The neurocrine pathway that acts through the transmitters such as acetylcholine.
 2. The endocrine pathway delivers hormones such as gastrin.
 3. The paracrine pathway delivers tissue factors, such as histamine, which are released from local storage sites.
- Thus, in intact mucosa, the parietal cells are exposed to many chemical messengers that may modulate their function. In the regulation of acid secretion, these various pathways are interdependent.

Parietal cells have receptors for several stimulant of acid secretion. They respond to two major signaling pathways acting via histamine and acetylcholine. Pharmacologically typical H₂-receptors have been demonstrated on human parietal cells. Combination of histamine with gastrin and acetylcholine causes potentiating effect on parietal cell function.

The tubulovesicular and canalicular structure of the parietal cells possess a specific hydrogen-potassium-ATPase enzyme (proton pump), which is responsible for the exchange of H⁺ or K⁺ ions across the apical surface.

The final process of acid transport per se rests with this enzyme. It is activated by protein kinases, histamine, acetylcholine and gastrin. The fundic mucosa also contains several paracrine cells, such as those containing somatostatin, histamine and serotonin (5-HT). They possess several receptors that may regulate acid secretion by modulating the release of the paracrine transmitters. Thus, acetylcholine may enhance the secretion of acid not only by stimulating the parietal cell directly, but also by reducing the level of somatostatin, a potent inhibitor of acid secretion.

Prostaglandin E (PGE) is produced by cell throughout the gastrointestinal tract (G.I. tract). Prostaglandin E (PGE) inhibits the secretion of gastric acid in humans, particularly food and non-steroidal anti-inflammatory drugs (NSAID) stimulated acid secretion. The pyloric glands, present in pyloric antrum, secrete gastrin directly into the blood and an alkaline, viscid, mucousrich juice, into the stomach. Gastrin stimulates acid secretion mainly by causing the release of histamine from the ECL (Enterochromaffin Like) cells, which are the sole source of gastric histamine involved in acid secretion.

The chief cells of gastric gland secrete pepsinogen, which is activated at acidic pH below 6 to the enzyme pepsin. Optimal activation occurs at pH 2. Enzyme and acid secretion in the stomach generally varies in parallel manner.

Normal gastric secretion acts as a chemical barrier to the bacterial invasion and is important for the maintenance of Introduction optimum pH at 1.5 to 4, necessary for the activity of pepsin, which is markedly reduced at pH above 4 and almost ceases at pH 5. 1.2.2 Phases of gastric secretion

Gastric acid secretion is generally divided into four phases:

Interdigestive or Basal phases

Cephalic phases ·

Gastric phases

Intestinal phases

During the **first three** phases, acid output is stimulated when food is first encountered and it continues as nutrients traverse the small intestine.

During the **fourth phase**, acid is secreted in the absence of an external stimulus, although the acid output can be affected by a person's emotional state.

Basal secretion This appears to follow a circadian rhythm, reaching its peak around midnight and its nadir at approximately 7 a.m. It is measured as nocturnal acid secretion, is high in some patients with duodenal ulcer but may be normal or even low in patients with gastric ulcer.

Secretion in response to food Gastric secretion in response to food may be divided into two phases, neurogenic and hormonal.

In neurogenic or cephalic phase The secretion occurs as a result of sight, smell, taste or even simple thought of food. The juice secreted is highly acidic and rich in pepsin. This secretion is Introduction mediated by the vagus and is abolished by vagotomy and muscarinic blocker by anticholinergic drugs. Alteration in the gastric secretion induced by emotions is also mediated centrally. In man, depression and fear result in a suppression of gastric secretion, where as anger and resentment result in increased production. Violent emotions cause congestion and hyperemia of the gastric mucosa, rendering it more susceptible to traumatic ulceration.

In hormonal phase (gastric and intestinal phase) The gastric secretion is increased due to stimulation of the parietal cells by gastrin. Gastrin containing cells predominantly located in the antrum are stimulated by food in the stomach and by neuronal input to release gastrin. Gastrin is carried to the ECL (Entero Chromaffin Like) cells where it stimulates the release of histamine, which then activates the H₂-receptors in the parietal cells.

In addition, gastric acid secretion is stimulated chemically, specifically by increases in intraluminal pH and by certain substances in the food, principally protein particularly peptic hydrolysates and individual amino acids. A cocktail are a protein broth soup before a meal stimulates acid secretion and prepares the stomach for the main dish. Digested protein in the duodenum enhances the output of acid. After a meal, the secretion of acid is modulated by a negative-feedback mechanism in which antral acidification inhibits the further release of gastrin. As the luminal pH approaches 3.5 to 3.0, the inhibition of gastrin becomes apparent and at pH 1.5 gastrin releases is completely inhibited. It is suggested that somatostatin, not only inhibits the antral release of gastrin but may also decreases acid secretion directly. Somatostatin and its analogue octreotide are potent inhibitors of gastric acid secretion and gastrin release. In addition, like gastric motility, gastric secretion is inhibited by reflexes initiated in the duodenum by distention, hypertonic content, fatty acids, amino acids acid in the duodenum.

Thus, high fat content of meal may delay gastric emptying to 4-6 hours, whereas a meal rich in carbohydrate and proteins may leaves the stomach in 2-3 hours.

Fat in the duodenum acts as a stimulus for the release of gut hormone cholecystokinin (CCK) where as acid in the duodenal chyme is an effective chemical stimulus for the release of another gut hormone secretin. Both secretin and cholecystokinin (CCK) act via circulation to inhibit the stomach motility and glandular secretion. The hormonal phase is inhibited only partially by anticholinergic drugs but substantially by H₂-receptor antagonist.

Natural gastrin is a polypeptide, containing 17 amino acids. A synthetic pentapeptide, pentagastrin, containing five amino acids (alanine, tryptophan, methionine, phenylalanine and aspartic acid) produces secretory and other biological effects similar to those of the natural gastrin. Histamine stimulated gastric secretion is highly acidic but poor in pepsin content. This is similar to gastrin-induced secretion since gastrin acts through the local liberation of histamine. It directly stimulates the parietal cell H₂-receptors that are linked to adenylyl cyclase.

Acetylcholine and gastrin also stimulate the receptors; however, histamine is thought to sensitize the parietal cells or to amplify the intracellular signals generated by these two secretagogues. Thus, H₂-receptor antagonists inhibit not only gastric secretion elicited by injected histamine but also that elicited by various physiological stimuli (whether mediated by the vagus or by gastrin). Adrenal glucocorticoids probably play a permissive role in the gastric acid secretion. Factors which are postulated to protect the gastric and duodenal mucosa against the effects of the 'aggressive factors' are as follows:

- Mucus produced by the cells of the gastric mucous glands, which forms a thin protective layer on the gastric mucosa.

Bicarbonate secreted by the surface epithelial cells, which protects the gastric mucosa from the effects of the highly acid gastric luminal contents.

Prostaglandins which enhance all the normal gastro duodenal protective mechanisms. Various growth factors such as epidermal growth factor, and transforming growth factor. A competent pyloric sphincter which prevents the regurgitation of the aggressive factors (bile acids and pancreatic enzymes) into the stomach.

No abnormalities have been consistently demonstrated in all patients with either duodenal or gastric ulcers, although various abnormalities have been demonstrated, each in some peptic ulcer patients these are:

Acid hyper secretion (basal and nocturnal) found in 30-40% of the patients with duodenal ulcer; it is seen only in patients with gastric ulcer due to Zollinger-Ellison syndrome.

Hereditary factors such as rapid gastric emptying and a larger than normal parietal cell mass found in peptic ulcer patients with rare genetic syndromes. Diminished gastric mucosal blood flow believed to be responsible for the acute gastric erosion occurring during serious medical or surgical illness (stress ulcers). An incomplete pyloric sphincter found in some patients with peptic ulcer.

High gastric acid output in stressful situations (such as an interview or an examination) and during certain emotions such as hostility, resentment, guilt or frustration. Their exact role in the average patient with peptic ulcer remains to be established.

Exogenous factors such as cigarette, smoking, NSAIDS, glucocorticoids and alcohol have been more strongly associated with ulcerogenesis, and non-healing of peptic ulcers (Satoskar et al., 2003). Introduction Currently treatments for peptic ulcers include antacids (systemic and non-systemic) acid secretion such as H₂ anti-histaminics, proton pump inhibitors, anticholinergics, ulcer protectives, ulcer healing drugs and anti-H.pylori drugs (Rang; 2003).

These drugs have decreased the morbidity rates, but produce many adverse effects including relapse of the disease, and are often expensive for the poor (Srikanta; 2007). In light of the above, it is pertinent to study natural products from plants as potential antiulcer compounds.

Due to less side effects compared to synthetic drugs, currently 80% of the world population depends on plant derived medicine for the first line of primary health care (Samy; 2007). The anti-ulcerogenic activity of many plant products is reported and have least side effects.

There are several plants used for gastric ulceration like *Tectona grandis*, *Azadirachta indica*, *Asparagus racemosus*, *Convolvulus pluricaulis*, *Embllica officinalis*, *Bacopa monniera*, *Withania somnifera* (Goel; 20028), which have protective effects against drug induced gastric mucosal injury, through different methods like Cold restraint induced ulcers (CRU), Pylorus-ligation method(PL method), Ethanol-induced method(EtOH method), Indomethacin induced method(INDO method)

1.4 Plant profile of *Musa sapientum*

Drug consists of dried leaves of *Musa sapientum* (**Family: Musaceae**), commonly known as **kela**, is a treelike perennial herb that grows 5 - 9 m in height, with tuberous rhizome, hard, long pseudostem.

The inflorescence is big with a reddish-brown bract and is eaten as vegetables. The ripe fruits are sweet, juicy and full of seeds and the peel is thicker than another banana.

It grows in humid lowland to upland tropical areas. Banana is a familiar tropical fruit. From its native Southwestern Pacific home, the banana plant spread to India by about 600BC and later on it spread all over the tropical world. It is possibly the world's oldest Introduction cultivated crop.

Taxonomical Classification

Kingdom : Plantae

Division : Magnoliophyta

Class : Liliopsida

Order : Zingiberales

Family : Musaceae

Genus : *Musa*

Species : *Sapientum*

Cultivation and Distribution

In different countries about 300 varieties of bananas are grown, of which a vast majority have been growing in Asian, Indonesia, Mexico, Costa, Rica, Columbia, Thailand are the top banana producing countries. It is extensively grown and cultivated as a fruit plant all over Bangladesh. The banana grows all most every where in the country throughout the year, mostly in Tamil Nadu, Maharashtra, West Bengal, Gujarat and Bihar. Tamil Nadu is the top banana producing state in India.

Leaves are large in size varies mostly with length about 75-80cm, width about 30-35cm and petiole is 8.5cm and midrib is so prominent on lower surface. Upper surface is glossy, smooth and wavy entire margin, acuminate apex with parallel venation.

Bracts are large opening in succession, ovate, 15-20 cm in length width at base is 6.6 cm, in middle 9cm, Introduction concave, dark red /dark brown in color and fleshy. Each bunch contains 13-17 bracts before the end of stalk.

Flowers are arranged radially on flowering stalk and covered by a bract. About 11-13 flowers are produced per cluster that are covered by bract and are individually attach with base. Each flower covering contain 5 small flowers and one large flower. Each flower has 7.3-7.8 cm length. (Zahid et al., 2015)

Microscopic Character of *Musa sapientum*

Bract reveals the presence of Spiral vessels with Phloem Fibres, Blackish-Brown pigmented thick-walled cells with spiral vessel and epidermal tissues with Stomata, Calcium oxalate Crystals, Nonglandular trichomes and Part of annual vessels along with group of Xylem Fibres are also present.

Powder of flower contains Fibro-vascular tissues, Groups of pigmented lignified cells containing Starch Grains, Epidermal tissues with stoma, Prism like Calcium oxalate. (Zahid et al., 2015)

Chemical constituents of *Musa sapientum*

Musa sapientum contains cycloartane triterpenes such as 3-epicycloeucalenol, 3-epicyclomusalenol, 24-methylenepollinastanone, 28norcyclomusalenone, 24-oxo29-orcycloartanone, cyclomusalenol, cyclomusalenone, 24-methylenecycloartanol, stigmast-7-methylenecycloartanol, stigmast-7-en-3-ol, lanosterol and β -amyirin.

It also contains several flavonoids and related compounds like leucocyanidin, quercetin and its 3-o-galactoside, 3-o-glucoside and 3-o-rhamnosyl glucoside.

It Introduction also contains acyl steryl glycosides such as sitoindoside-I, sitoindoside-II, sitoindoside-III, sitoindoside-IV and steryl glycosides such as sitosterol gentibioside, sitosterol myo-inosityl- β -D-glucoside.

It also contains Vit. C, Vit. B, mineral salts and fats.

Carbohydrates, cellulose, hemicelluloses, arginine, aspartic acid, glutamic acid, leucine, valine, phenylalanine and threonine have been isolated from pulp and peel of *Musa* (Ketiku, 197315 & Emaga et al., 2007)

Traditional Uses

FRUIT

Used in diarrhea, dysentery, intestinal lesions in ulcerative colitis, diabetes, in uremia, nephritis, gout, hypertension, cardiac disease (Ghani; 20037 and Khare; 2007).

Leaves

Used as cool dressings for blister and burns (Ghani; 2003).

Flower

Used in dysentery, diabetes and menorrhagia.

Stem

Used in cholera, otalgia and haemoptysis.

Root

Used as anthelmintic (Khare; 200716), blood disorders, venereal disease (Ghani; 2003).

Reported Activities

Anti-ulcer activity (Rabbani et al., 1999), Antimicrobial activity (Fagbemi et al., 2009), Hypoglycemic activity (Ojewole and Adewunmi; 2003), Hypocholestromic activity (Usha et al., 1984, Antihypertensive activity (Osime et al., 1990), Antioxidant activity (Yin et al., 2008), Diuretic activity (Jain et al., 2007).



Figure 1: Image showing plant of *Musa sapientum*

1.5 Plant profile of *Phoenix dactylifera*

Drug consists of dried leaves of *Phoenix dactylifera* (**Family: Arecaceae**), also known as date palm, is a monocotyledon plant within the palm fruit tree family and they can be found mostly in the North Africa and Middle East region (Baliga et al., 2011).

Phoenix dactylifera is a palm in the genus *Phoenix*, cultivated for its edible sweet fruit. Although its place of origin is unknown because of long cultivation, it probably originated from lands around the Persian Gulf. It is believed to be indigenous to the countries around the Arabian Gulf. Different parts of the plant are traditionally claimed to be used for the treatment of Introduction a broad spectrum of ailments including memory disturbances, fever, inflammation, paralysis, loss of consciousness and nervous disorders (Pujari et al., 2014).

Taxonomical Classification Kingdom :

Plantae Division : Angiosperms

Class : Monocots

Order : Arecales

Family : Arecaceae

Genus : *Phoenix*

Species : *Dactylifera*

Cultivation and Distribution

Phoenix dactylifera Linn. propagated by seed or off-shoots. Seedlings are first planted in nursery rows and later transplanted to their permanent location. Sometimes planted around Aswan, they plant several seedlings in the same hole to insure the presence of females. Normal healthy trees may produce 10-30 or more offshoots. These will root if their bases are encased in soil. In Egypt they say it takes 8 years for an offshoot to yield economically. Not known in the wild state, cultivated and sub spontaneous throughout the desert regions between 15° and 35° C, from the Canaries and Morocco in the west to India in the east. It is recorded from all the part from Syria, Palestine, Transjordan, Iraq, Arabia, Iran, and Baluchistan. Cultivated as an ornamental in South Europe, but seldom matures fruit except in extreme southern parts of Italy and Spain. It is also

cultivated in Arizona and California, USA and Queensland, Australia. There is evidence of date usage 8000 years ago in W. India as well as its cultivation in Sumeria and the ancient Egyptian empire (Knight, 198017)

Macroscopic Character of Phoenix dactylifera

The date palm is dioecious having separate male and female plants. They can be easily grown from seed, but only 50% of seedlings will be female and hence fruit bearing, and dates from seedling plants are often smaller and of poorer quality. The date is an erect palm to 100 or 120 ft (30.5-36.5 m), the trunk clothed from the ground up with upward-pointing, overlapping, persistent, woody leaf bases. After the first 6 to 16 years, numerous suckers will arise around its base. The feather-like leaves, up to 20 ft (6 m) long, are composed of a spiny petiole, a stout midrib, and slender, gray-green or bluish-green pinnate 8 to 16 in (20-40 cm) long, and folded in half lengthwise. Each leaf emerges from a sheath that splits into a network of fibers remaining at the leaf base. Small fragrant flowers (the female whitish, the male waxy and cream colored), are borne on a branched spadix divided into 25 to 150 strands 12 to 30 in (30-75 cm) long on female plants, only 6 to 9 in (15-22.5 cm) long on male plants. One large inflorescence may embrace 6,000 to 10,000 flowers.

Chemical constituents of Phoenix dactylifera

It contains carbohydrates, alkaloids, steroids, flavonoids, vitamins and tannins. It also contains 5-o-caffeoylshikimic acid also called as dactyliferic acid. It also contains flavonoid glycosides like luteolin, methyl luteolin, luteolin-7-glucoside, luteolin-7-rutinoside, glycosylapigenin, quercetin, methyl quercetin and flavanols like catechin & epicatechin. Pollen contains cholesterol and rutin.

The hemicellulose of the pollen contains 46% arabinose, 25% galactose, 18% xylose, 9% rhamnose, and 2% uronic acid.

Seeds contain cholesterol estrones, polysaccharide A (with 10:1 D-mannose: D-galactose) polysaccharide B and xylose.

The fruit pulp also contains leucanthocyanins, pipercolic acid, 5-oxypipercolic acid (C₆H₁₁NO₃) and the piperidine derivative baikiaian (C₆H₉NO₂) as well as tannin. (Pujari et al., 2011)

Traditional Uses

Fruit

Used in ulcer, used as a hepatoprotective drug, in diarrhea, as anti-inflammatory and antioxidant activity (Pujari et al., 2011)

Leaves

Used in Diabetes (Mard et al., 2010)

Reported Activities

Antirolithiatic activity (Reddy et al., 2013), Antioxidant and Antimutagenic activity (Vayalil; 2002), Anticancer activity (Ishurda et al., 2005), Antidiabetic and Antilipademic activity (Mard et al., 2010).



Figure 2: Image showing plant of *Phoenix dactylifera*

2.1 LITERATURE REVIEW

Jainu M et al (2005) studied antiulcerogenic and ulcer healing effects of *Solanum nigrum* (L.) on experimental ulcer models. They investigated the antiulcer effect of *Solanum nigrum* fruits extract (SNE) on cold restraint stress (CRU), indomethacin (IND), pyloric ligation (PL) and ethanol (EtOH) induced gastric ulcer models and ulcer healing activity on acetic acid induced ulcer model in rats. The treatment with SNE at higher dose significantly inhibited the gastric lesions induced by CRU (76.6%), IND (73.8%), PL (80.1%) and EtOH (70.6%), respectively, with equal or higher potency than omeprazole.

Odabasoglu F et al (2006) studied the gastroprotective and anti-oxidant effect of usnic acid on indomethacin-induced gastric ulcer in rats. The gastroprotective effect of usnic acid (UA) isolated from *Usnea longissima* was investigated in the indomethacin induced gastric ulcers in rats at doses of 25, 50, 100 & 200 mg/Kg body weight. The gastric lesions were significantly reduced by all doses of UA as compared with indomethacin (25mg/Kg body weight) treated group. In the stomach tissues of treated animals, the in-vivo antioxidant level was evaluated. The administration of indomethacin caused a significant decrease the Literature Review levels of superoxidase dismutase (SOD), glutathione peroxidase (GPx) and reduced glutathione (GSH) and increase in the lipid peroxidation (LPO) level ($p < 0.05$) and reserpine-induced ($P < 0.01$) models in rats.

Ray S (2007) studied the acute p.o. administration of absolute ethanol (1.0 ml/kg) to fasted rats produced extensive necrosis of gastric mucosa. Pretreatment with p.o. administration of Propolis ethanol extract (PEE) could effectively and dose-dependently prevent such necrosis. This protective effect is called "cytoprotection". The maximal cytoprotective effect against absolute ethanol (AE)- induced gastric mucosal lesion was observed 1 hour after propolis extract administration. A gross examination of the gastric mucosa showed a marked improvement in groups receiving PEE. In order to further investigate the gastric protective mechanism of propolis, lipid peroxidation (LPO) levels in vivo and in vitro were estimated.

Ray S (2008) studied that the prevention of experimentally-induced gastric ulcers in rats by an ethanolic extract of "Parsley" *Petroselinum crispum*. Ethanolic extract of *Petroselinum crispum* Mill. (Umbelliferae), was tested for its ability to inhibit gastric secretion and to protect gastric mucosa against the injuries caused by pyloric ligation, hypothermic restraint stress, indomethacin and cytotoxic agents (80% ethanol, 0.2 M NaOH and 25% NaCl) in rats. The extract in doses of 1 and 2 g/kg body weight had a significant antiulcerogenic activity on the models used.

Ray S (2009) studied that the Guarana (*Paullinia cupana* Mart.) offers protection against gastric lesions induced by ethanol and indomethacin in rats. The effects of guarana (*Paullinia cupana*) extract were analyzed in rats on acute gastric lesions induced by ethanol and indomethacin and were compared to those produced by caffeine, a methylxanthine. Guarana (50 and 100 mg/kg p.o.) pretreated animals showed a significant reduction in the severity of gastric lesions induced by absolute ethanol in a manner similar to caffeine (20 and 30 mg/kg p.o.). Against indomethacin-induced gastric ulceration, guarana at a higher dose offered Literature Review significant protection but caffeine was ineffective at the doses tested. In 4 h pylorus-ligated rats, both guarana and caffeine caused significant diminution in the gastric secretory volume as well as the total acidity.

Ray S (2010) studied the healing effects of Gotu kola water extract and asiaticoside, an active constituent of Gotu kola, on acetic acid induced gastric ulcers (kissing ulcers) in rats were examined. Gotu kola was prepared from *Centella asiatica* dry plant and the concentration of asiaticoside in Gotu kola was quantitatively determined with the use of high performance liquid chromatography analysis. Different concentrations of Gotu kola and asiaticoside were orally administered to rats with kissing ulcers. They were found to reduce the size of the ulcers at day 3 and 7 in a dose-dependent manner, with a concomitant attenuation of myeloperoxidase activity at the ulcer tissues.

Qin Z et al (2011) studied that the synergistic action of famotidine (FMD) and chlorpheniramine (CPA) on acetic acid-induced chronic gastric ulcer in rats. Chronic gastric lesions were induced in male Sprague-Dawley (SD) rats by serosal application of the acetic acid. Forty SD rats were randomly divided into blank group (n = 8), control group (n = 8), FMD group (n = 8), CPA group (n = 8), and FMD & CPA group (n = 8). Each group was given intraperitoneally (i.p.) 0.5 mL/100 g distilled water, 9 g/L NaCl saline, 4 mg/kg FMD, 10 mg/kg CPA, 4 mg/kg FMD & 10 mg/kg CPA, respectively, daily for 10 days. On day 10, ulcer area was determined by planimetry. The level of myeloperoxidase (MPO) in the liver homogenization was determined by biochemical methods and the plasma levels of 6-ketoprostaglandin F1 alpha and IL-8 were determined by radioimmunoassay.

Mishra A et al (2011) investigated that the diuretic activity of *Musa sapientum* L. (family: Musaceae) flowers. The dried powder of the flower was subjected to Soxhlet extraction with alcohol and this extract was used for diuretic activity in Wistar albino rats using Lipschitz method.

Prabha P et al (2012) discussed that Peptic ulcer disease (PUD), encompassing gastric and duodenal ulcers is the most prevalent gastrointestinal disorder. The pathophysiology of PUD involves an imbalance between offensive factors like acid, pepsin and defensive factors like nitric oxide and growth factors. The clinical evaluation of antiulcer drugs showed tolerance, incidence of relapses and side-effects that make their efficacy arguable.

Adewoye E O et al (2013) was carried out to investigate the effect of methanolic extract of *Musa sapientum* leaves (MEMSL) on gastrointestinal transit time (GITT) in male albino rats with and without hyperglycaemia and to elucidate possible mechanism by which this extract functions. The study showed significant decrease in GITT in the normal rats treated with 250mg/kg and 500mg/kg of extract.

Agrawal P K et al (2014) reported that *Musa sapientum* has been shown to possess wound healing activity.

Alisi C S et al (2015) has been reported that the aqueous extract of unripe fruit peels and leaves of *Musa sapientum*, show antimicrobial activity against *Staphylococcus* and *Pseudomonas* species in dehydrogenase assay. The IC₅₀ of the aqueous fruit peel extract were 143.5 and 183.1 µg/ml against *Staphylococcus* and *Pseudomonas* species respectively and in case of leaf extract were 401.2 and 594.6 µg/ml respectively.

Vijayakumar S et al (2016) reported the antioxidant activity of the extracted flavonoids from *Musa paradisiaca* in rats. They found that the flavonoids from banana stimulated the activities of superoxide dismutase (SOD) and catalase which might be responsible for the reduced level of peroxidation products such as malondialdehyde, hydroperoxides and conjugated dienes.

Kaou A M et al (2017) reported that the decoction of the leaves of *Musa paradisiaca* added to *Ocimum americanum* and *Ocimum gratissimum* is used as to Literature Review treat malarial in Comores, Ngazidja. But in vitro study using *Plasmodium falciparum* chloroquine-resistant strain proves this plant ineffective in malaria.

Andrade C U B et al (2018) reported the mutagenic effect of *Musa paradisiaca* fruit peel extract in mice assessed by the single-cell gel electrophoresis (SCGE) and micronucleus assays. The experiments showed DNA damaging property in peripheral blood leukocytes for 1500 and 2000 mg/kg body weight.

Tewtrakul S et al (2019) has been reported that the water extract of pulp of ripe *Musa sapientum* have significant anti-allergic activity on antigen induced degranulation in RBL-2H3 cells with an IC₅₀ value of 13.5±2.4. Jain D L et al (2007) reported that the Ash of the peel of *Musa sapientum* showed an increase in urine volume and K⁺ as well as other electrolyte excretion than normal saline in a study in rats. Successive ethanolic extract also give this diuretic effect.

Mallick C et al (2020) reported that the methanolic root extract of *Musa paradisiaca* showed total cholesterol (TC), triglyceride (TG), LDLc and VLDLc lowering effect in diabetic rats.

Mokbel M S and Hashinaga F (2020) was reported that the aqueous acetone extract of banana peel by β-carotene bleaching method, DPPH free radical scavenging and linoleic acid emulsion method. Glycosides and monosaccharide components are mainly responsible for the antioxidant activity.

Dhanabal S P et al (2021) reported that the antihyperglycemic effect of ethanolic extract of flowers of *Musa sapientum* (Musaceae), in alloxan induced diabetic rats.

Lewis D A and Shaw G P (2001) reported that the leucocyanidin and the synthetic analogues, hydroxyl ethylated leucocyanidin and tetra allyl leucocyanidin, found to protect the gastric mucosa in aspirin-induced erosions in rat by increasing gastric mucus thickness.

Rabbani G H et al (2021) reported that the antidiarrhoeal activity of banana in rats. The antidiarrhoeal activity of green banana diet was found very effective in children with diarrhoea. 2.3 Literature Review for *Phoenix dactylifera* Linn.

Zakaria A S et al (2022) reported that *Phoenix dactylifera* date palm shows in vivo antioxidative and hepatoprotective effects.

Najat A B and Kahkashan P (2022) reported that the Antifungal potential of water, acetone and methanol extracts of leaves and pits of *Phoenix dactylifera* L. var.

Mard S A et al (2022) reported that leaves of Phoenix dactylifera shows antidiabetic activity and antilipademic activity.

Ahmed S H and Rocha J B (2022) showed in your study that the antioxidant activities (AA %) of the water extract for Phoenix dactylifera, Loranthus europeas, Zingiber officinalis, Citrus aurantifolia was measured by the TBARS method. Total phenol content, DPPH scavenger free radical activity and the iron chelation capacity of these extracts were also quantified.

Mohamed B A et al (2023) reported that the protective effects of extract from dates and ascorbic acid on Thiocetamide induced hepatotoxicity in rats.

Burgoyne R W and Tan D H (2023) reported that the prolongation and quality of life for HIV infected adults treated with highly active antiretroviral therapy a balance act.

Ishurda O et al (2023) reported that the anticancer activity of polysaccharide prepared from Phoenix dactylifera.

Al-Qarawi A A et al (2023) showed that the present work aimed at testing, in a rat model of ethanol induced gastric ulceration, a local folk medicinal claim that dates are beneficial in gastric ulcers in humans.

Vayalil P K (2023) reported that the fruit of Phoenix dactylifera shows antioxidant and antimutagenic activity.

Adewoye E.O., Ige A.O. and Latona C.T.(2024), Effect of Methanolic extract of Musa sapientum leaves on Gastrointestinal Transit time in Normal and Alloxan induced Diabetic rats: Possible Mechanism of Action, Niger J Physiol Sci., Nov 23;26(1): pp 83-88.

Adeyemi E. O., Bastaki S.A., Chandranath I.S., Hasan M.Y., Fahim M., and Adem A. (2024), Mechanisms of action leptin in preventing gastric ulcer, World J.Gastroenterol., Jul 21, 11(27): pp 4154-60.

Agarwal P.K., Singh A., Gaurav K., Goel S., Khanna H.D. & Goel R.K., (2024), Evaluation of wound healing activity of extracts of plantain banana(Musa sapientum var.paradisica) in rats, Indian Journal of Experiment Biology, Januray,Vol.47: pp 32-40. 4.

3.1 AIM & OBJECTIVES

3.2 PHARMACOGNOSTIC STUDIES OF PHOENIX DACTYLIFERA

To study the pharmacognostic features of Phoenix dactylifera.

To carry out material & method plan.

To study the macroscopic & microscopic characteristics of Phoenix dactylifera.

To study the evaluation parameter of Ash value, % extractive value & powder analysis of Phoenix dactylifera.

3.3 PHYTOCHEMICAL STUDIES OF MUSA SAPIENTUM

To study the phytochemical Studies on Leaves of Musa sapientum.

To carry out the sample preparation.

To carry out the extraction methods.

To carry out the tests to identify the different group of compounds present in different extracts.

To carry out the thin layer chromatography study of different extracts.

To carry out the High-Performance Thin layer chromatography study of different extracts.

To carry out the Isolation and characterization of compounds by IR, NMR and Mass from ethanolextract of *Musa sapientum*.

To carry out the Isolation and characterization of compounds by IR, NMR and Mass from chloroform extract of *Musa sapientum*.

4.1 MATERIAL & METHOD

4.1.1 PHARMACOGNOSTIC STUDIES OF PHOENIX DACTYLIFERA

4.1.2 INTRODUCTION

Pharmacognosy is the study of natural substances, principally plants; those have got their use in medicine. Pharmcognosy encompasses also the knowledge of the history, distribution, cultivation, collection, processing for market and preservation, the study of organoleptic, physical, chemical, and structural characters and the uses of crude drugs (Trease et al., 1997).

The objective of pharmacognosy is to contribute to rational relationship between Chemical moieties of naturally occurring drugs and their biological and therapeutic effects.

The macroscopic description includes shapes, size, character of outer and inner surface, type of fracture and organoleptic characters like colour, odour, taste etc. Some more characters are identified microscopically to judge whether the powdered drug is coming from the definite source.

In addition, other properties like ash value values, extractive values, behavioral pattern with different chemical reagents and fluorescent characteristics on exposure to U.V. light as well as qualitative chemical tests etc. are performed for identification and authentication of the crude drugs. (Ghosh, A.K., 20131)

4.1.3 Plant materials

The fresh *Phoenix dactylifera* leaves were collected from local farmers in the Rohilkhand region .

The leaves were dried in shade and powdered by using laboratory grinder. The powder to pass through 40 no.mesh sieve and stored in closed vessel.

The intact leaves were taken for evaluation of macroscopic character and powder of leaves was accepted for several other studies.

4.1.4 Macroscopic Description

This evaluation tells us about the morphological and sensory profiles of the crude drug, the leaves of the plant *Phoenix dactylifera* (Wallis, T.E., 1976). The following is the result:

1. Habitat: Tree (Dioecious plant)
2. Arrangement: Overlapping, upward pointing and persistent
3. Shape: Flattened

4. Size: Length-about 110-145cm with 140-160 leaflets
5. Leaflet size: Length-about 12-30cm and width-about 0.8-2.0 cm
6. Base: Having petiole with spine and woody leaf bases
7. Midrib: Not distinguished properly
8. Apex: Acute with sharp spine
9. Margin: Entire
10. Venation: Parallel
11. Texture: Rough and smooth
12. Fracture: Fibrous
13. Colour: Dark green (Both surfaces)
14. Odour: Distinct
15. Taste: Astringent and acrid
16. Lamina: Linear-lanceolate
17. Surface: Glabrous

4.1.5 Microscopic character

The transverse section of leaf is stained with 0.1% (w/v) toluidine blue in lactic acid. Transverse sections showed the typical monocot tissue anatomy with parenchyma and vascular tissue particularly apparent

Parenchymatic cells often showed starch grains (arrowheads) and calcium oxalate crystals (arrow).

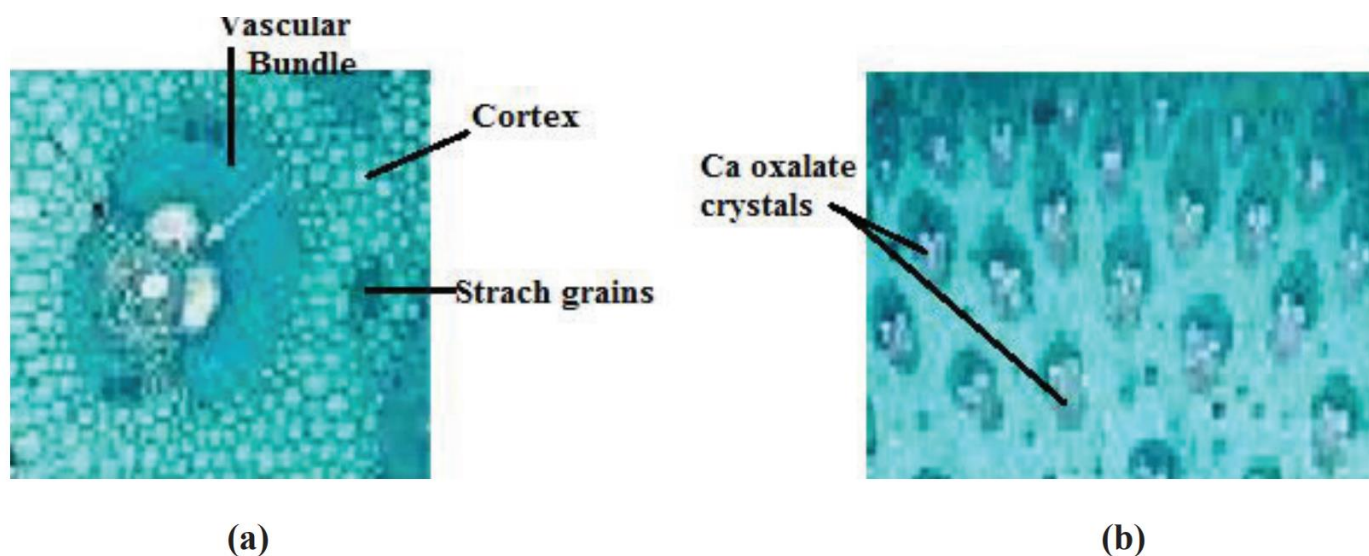


Figure 3: T.S of Phoenix dactylifera leaf (a) Starch grains (b) Ca oxalate crystals

4.1.6 Ash value evaluation

Different ash values were determined by conventional methods and reported in Table No.4.1 (Kokate et al., 1999).

Procedure for total Ash



Figure 4: Procedure for total Ash

Procedure for water soluble Ash

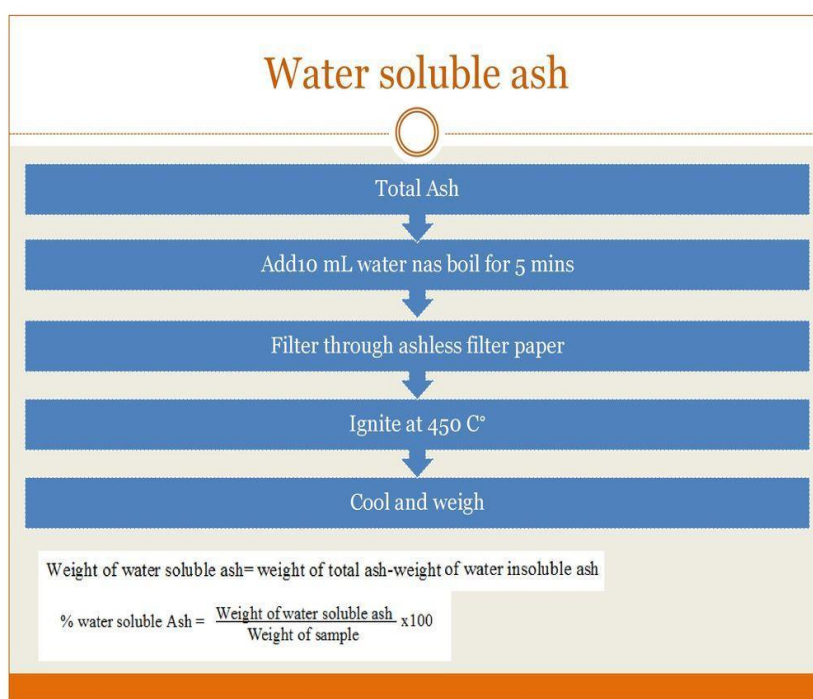


Figure 5: Procedure for water soluble Ash

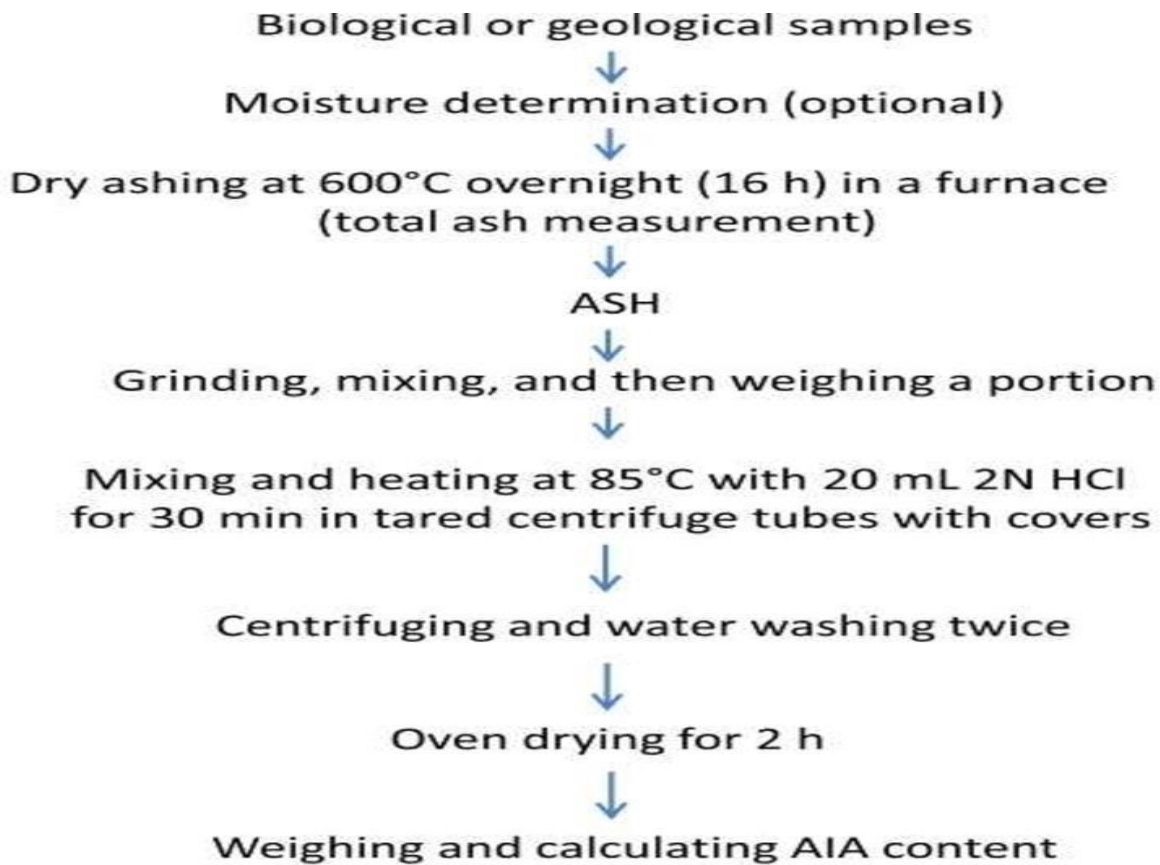
Procedure for Acid soluble ash**A new method for measuring acid insoluble ash (AIA)**

Figure 4.4: Procedure for Acid soluble ash

PROCEDURE FOR WATER INSOLUBLE ASH

Extracting total ash with hot water & filtered.



The residue is dried and ignited to give the water-insoluble ash.



The water-soluble ash is obtained by difference from the total ash

Figure 6 : Procedure for Acid soluble ash

PROCEDURE FOR ACID INSOLUBLE ASH

Dissolving ash in dilute hydrochloric acid (10% m/m).



The liquid filtered through an ashless filter paper and thoroughly washed with hot water.



The filter paper is then ignited in the original dish, cooled and weighed.

Figure 7 : Procedure for Acid soluble ash

Table 1: Ash value of powdered leaves of Phoenix dactylifera

Type of Ash	Percentage (w/w)
Total Ash	14
Water soluble ash	94.55
Acid soluble ash	96
Water insoluble ash	5.45
Acid insoluble ash	4

4.1.7 Percentage (%)-Extractives evaluation

Consider the diversity of chemical nature and properties of contents of drugs. Various solvents are used for determination of extractives. The quantity of solvents utilized to dissolve appreciable quantities of substances and the %- extractive values are calculated and the reported in the Table No.4.2 (Kokate et al., 2012).

4.1.8 Procedure for Extraction Via Soxhlet Apparatus

Solvent extraction is done in a soxhlet apparatus to extract the chemical constituents from leaves.

This particular soxhlet apparatus consists of a glass extractor, fitted in between a round bottom flask at the bottom and a bulb condenser at the top.

Inside the glass thimble holder, solid matrix of leaves is placed within thimble.

The round-bottom distillation flask initially contained an extracting solvent and it is heated up by electrothermal heating mantle 450 C° maximum temperature, 1L max capacity and power 300W .

As the solvent vapor goes up to the condenser, it condenses and accumulates inside the extractor.

Here, the solvent comes in contact with the leaves.

When the condensate moves down through the bed of leaves, mass transfer takes place and accumulated solvent moves up purely due to the hydrostatic pressure head so, surface area offered by the bed and the leaves-solvent contact time are the two major factors for the yield of extraction.

Table 2 : Extractives of powdered leaves of *Phoenix dactylifera* in different solvents

Solvents used	%-Extractives (w/w)
Petroleum ether	3.45
Benzene	8.50
Chloroform	15.89
Acetone	10.35
Ethanol (95%)	18.40

4.1.9 Powder analysis

Table 3: Powder analysis of *Phoenix dactylifera*

Powder analysis of *Phoenix dactylifera*

Chemicals/Reagents	LongUV(365nm)	Short UV(254nm)	Visible Light
Powder drug (Without Chemicals)	Light Brown	Yellow	Green
NaOH (10% aq.)	Brown	Yellowish Brown	Green
Meth. NaOH (10%)	Yellowish Brown	Yellowish Green	Light Green
Conc. H ₂ SO ₄	Black	Yellowish Brown	Yellowish Green
Dil H ₂ SO ₄	Dark Brown	Yellowish Brown	Yellowish Green
Dil.HCl	Yellowish Brown	Yellow	Light Green
Conc.HNO ₃	Dark Brown	Yellowish Brown	Reddish Brown
Glacial Acetic acid	Brown	White	Yellowish Green
Ethyl Acetate	Yellowish Brown	Yellow	Greenish Yellow
Toluene	Yellowish Brown	Yellow	Yellowish Green
Ammonia Sol.	Dark Brown	Yellow	Yellowish Green

The analysis of powder/extract under daylight is unreliable due to lack of fluorescence. The colour characteristics were observed under day light and UV light.

4.1.10 Phytochemical Studies on Leaves of *Musa sapientum*

4.1.11 Introduction

The herbs are the biosynthetic laboratories where the herbs (or medicinal herbs or plants) produce and store some secondary metabolites. These secondary metabolites are several classes of organic chemical compounds e.g. alkaloids, amino acids, carbohydrates, fixed oils, flavonoids, glycosides, gums, resins, saponins, sugars, tannins, terpenoids, volatile oils, wax, etc.

Depending on the nature and quantity of these chemical compounds the herbs show their variations in medicinal activities. There are a large number of traditionally used herbs on earth having a wide range of medicinal efficacies.

After establishment of botanical identity preliminary phytochemical screening has done.

The screening thus involves the followings:

- i) extraction of the plant material;
- ii) separation and isolation of the constituent/s of interest;
- iii) characterization of the isolated compound/s of pharmaceutical importance. The herbal drug or herbal drug preparation is entirely regarded as the active substance. These constituents are either of known therapeutic activity or are chemically defined substances or groups of substances generally accepted as to contribute substantially to the therapeutic activity of any herbal drug.

Qualitative chemical examination is done to detect and isolate the active constituent/s. High performance liquid chromatography (HPLC) is one of the main analytical techniques for quantitative measurements of active compounds. In cases when active ingredients are not known or are too complex, the quality of plant extracts may be assessed by the "fingerprint" chromatogram (Trease & Evans, 1997).

The choice of extraction procedure depends on the nature of plant material and the components to be isolated. Dried materials are usually powdered before extraction, whereas fresh plants (leaves, etc.) can be homogenized or macerated with a solvent such as alcohol. The latter is also particularly useful for stabilizing fresh leaves by dropping them into the boiling solvent.

Alcohol is a general solvent for many plant constituents (most fixed oils excepted) and as such may give problems in the subsequent elimination of pigments, resins, etc.

Extraction itself may be performed by repeated maceration with agitation, percolation or by continuous hot extraction e.g. in a Soxhlet extractor. The plant extract is then concentrated in the vacuum evaporator, usually in a rotatory evaporator.

If a single component is present in the extract, the crystals may be purified by re-crystallization. In most of the cases, a mixture of components is present and it is necessary to re-dissolve them in a suitable solvent and is separated by chromatography. The concentrated extract, free from solvent, should be stored in a refrigerator.

Several techniques are applied for the isolation and characterization of medicinal compound/s from plant extract/s. However, a number of technological advances during the past few years have significantly replaced the earlier strategies for screening natural products.

In particular, combinatorial chemistry has provided an alternative means to increase sample diversity and advances in automation and instrumentation have enabled in-house compound libraries to be screened in a fraction of the time required only a few years ago.

The separation, isolation and purification of plant constituents are carried out using one or combination of different chromatographic techniques e.g. Paper chromatography (PC), Thin layer chromatography (TLC), which is now modified to High performance thin layer chromatography (HPTLC); Gas liquid chromatography (GLC) and Column chromatography (CC), etc.

The choice of technique largely depends on the solubility property and volatilization property of the compounds to be separated. Sometimes a combination of PC & TLC or TLC & GLC or HPTLC may be the best way for separation of a particular class of plant constituent.

Some of the chromatographic techniques generate quantities of material sufficient for a complete analysis by modern spectrometric methods e.g. UV/Vis spectroscopy, IR spectroscopy, NMR-spectroscopy, Mass-spectroscopy, etc (Harborne, J.B., 1973).

4.1.12 Preparation of the samples

The various extracts were made directly from authenticated crude plant material in powdered form. The compositions, quality and therapeutic effects differ from each other substantially. Standardized extracts are of high-quality extracts having consistent levels of specified compounds. The leaves of *Musa sapientum* having consistent levels of specified compounds. The leaves of *Musa sapientum* in powdered form were used for the present study.

4.1.13 Extraction methods

The choice of extraction procedure and solvents depend on the nature of the plant material and the component/s to be isolated.

4.1.14 Infusion

This method is used for those drugs which are soft in nature so that water may penetrate easily to the tissues and the active constituents are water soluble .

The simplest form of apparatus consists of a beaker or a tea pot but special pots known as infusion pots are also available. The drug to be extracted is placed at the bottom of the pot, water added and the contents stirred occasionally.

Otherwise the drug may be enclosed in a piece of muslin and suspended just below the level of the water. The drug is allowed to remain in contact with water for the required time, usually 15 mins. After the specified time, the liquid is strained and dispensed. The marc is not pressed to avoid expression of colloidal cells into the preparation and the final volume of the preparation is not adjusted by adding more of vehicle otherwise dilution of active constituents will take place. (Gupta, A.K., 1994).

4.1.15 Successive extractions of powdered leaves of *Musa sapientum* by Petroleum ether (60o -80oC), Benzene, Chloroform, Acetone and Ethanol (95%)

The powdered leaves of *Musa sapientum* was dried first at below 40oC for half an hour and then passed through 40no. mesh to get a uniform powder of crude drug.

50g of that powder was taken to extract successively with 500ml of Petroleum ether (60o -80oC), 500ml of Benzene, 500ml of Chloroform, 500ml of Acetone and 500ml of Ethanol in a Soxhlet apparatus.

After drying the petroleum ether extract, a dark green sticky mass was obtained. The mass was stored in refrigerator for future purpose. The marc was dried under controlled temperature and allowed for extraction by benzene in Soxhlet.

After drying the benzene extract, a yellowish brown coloured solid mass was obtained. The mass was stored in refrigerator for future use. The marc was dried under controlled temperature and allowed for extraction by chloroform in Soxhlet.

After drying the chloroform extract, a yellowish green coloured solid mass was obtained. The mass was stored in refrigerator for future use. The marc was taken out and dried under controlled temperature and then that was allowed for extraction by acetone in Soxhlet.

In the same manner as done in the above cases the extract was dried to get a mass of green coloured. That mass was stored in refrigerator for future testing. The marc was taken out and used to extract by ethanol in Soxhlet.

The ethanol extract was dried to get a dark brownish green coloured mass, which was stored in refrigerator for future use. The report of yield and results of phytochemical investigations are given in Table No.7 and 8.

4.1.16 Tests to identify the different group of compounds present in different extracts (Qualitative chemical examination).

The extracts obtained as above are then subjected to qualitative tests for the identification of various plant constituents.

Test for alkaloids

Stirred a small portion of the solvent free chloroform and alcoholic extracts separately with a few drops of dilute HCl and filter.

The filtrate may be tested carefully with various alkaloidal reagents such as Mayer's reagent (cream precipitate), Wagner's reagent (reddish brown precipitate), Dragendorff reagent (orange brown precipitate) and Hager's reagent (yellow precipitate) indicated the presence of alkaloids.

Mayer's reagent: 1.36g of mercuric chloride is dissolved in 60ml distilled water (A). 5g of potassium iodide is dissolved in 20ml distilled water (B). (A) and (B) are mixed and the volume is adjusted to 100ml with distilled water.

Dragendorff's reagent: 14g of sodium iodide is boiled with 5.2g basic bismuth carbonate in 50ml glacial acetic for a few minutes. It is allowed to stand overnight and filtered off the precipitate of sodium acetate crystals. To 40ml of the red-brown filtrate 160ml of ethyl acetate and 1ml water are added. The stock solution

is preserved in amber-coloured bottle. When needed, 20ml of acetic acid is added to 10ml of this stock solution and made upto 100ml with water

Hager's reagent: A saturated aqueous solution of picric acid used for detection of alkaloids.

Wagner's reagent: 1.27g of iodine and 2g of potassium iodide are dissolved in 5ml of water and made up the volume to 100ml with distilled water. It is used for detection of alkaloids. (Ghosh, A.K., 2013).

Test for flavonoids

Shinoda's Test: Extract was dissolved in ethanol, warmed and then filtered. Mg-chips (ribbons) were then added to the filtrate followed by few drops of concentrated HCl. Colour changing from pink, orange or red to purple indicated the presence of flavonoids. (Buvaneswari, K., 2011)

A little amount of the extract is digested with 10% v/v sulphuric acid, cooled and extracted with diethyl ether. The ether extract is divided into three portions. In a portion 1ml of 0.1N sodium hydroxide is added, in the second portion 1ml of diluted sodium bicarbonate solution is added and to the third portion 1ml of diluted ammonia is added.

Development of yellow colour in each case indicates the presence of flavonoids. (Ghosh, A.K., 2013)

Test for Saponins

Foam Test

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth, which indicates the presence of saponins. (Buvaneswari, K., 2011)

Test for steroids

Liebermann-Burchard Test

ml of acetic anhydride was added to the solution along with 2 ml of conc. H₂SO₄. The colour changed from violet to blue or green in some samples. This indicates the presence of steroid.

Test of Fixed Oils and Fats

A small quantity of extract (after removal of the organic solvent) is pressed in between the two filter papers. Oil stains on the paper indicate the presence of fixed oil.

A few drops of 0.5 (N) alcoholic potassium hydroxide is added to a small quantity of extract (after removal of the organic solvent) along with a drop of phenolphthalein. The mixture is heated on water bath for 1-2 hours.

Formation of soap or partial neutralization of alkali indicates the presence of Fixed Oils and Fats. (Ghosh, A.K., 2013)

Test for Terpenoids

Salkowski Test

5ml of each extract mixed with 2ml of chloroform, and 3 ml concentrated H₂SO₄ was carefully added to form a layer reddish brown colour of the interface was formed to show positive results for the presence of terpenoids. (Buvaneswari, K., 2011).

Test for Gums and Mucilage's

About 10ml of extract is added slowly to 25ml of absolute alcohol with constant stirring. The precipitate is filtered and dried in air. The precipitate is examined for its swelling properties and for the presence of carbohydrates. (Ghosh, A.K., 2013)

Test for Amino acid and Protein**Ninhydrin Test**

Take 2-3 ml of sample solution in a test tube. Add 3-4 drops of ninhydrine solution and heat. Appearance of purple or violet indicates the presence of proteins.

Test for carbohydrates**Benedicts test**

Add 1ml of Benedicts reagents to test tube and heat the mixture to boiling in a water bath for 2mins ,the formation of an orange red colour precipitate due to formation of a copper (I) oxide indicates the presence of reducing sugars.(Buvaneswari, K., 2011)

Molisch's test

Take sample solution in a test tube and add Molisch's reagent (10g of alpha-naphthol is dissolved in 100ml of 95% alcohol). It indicates purple colour due to presence of carbohydrates. (Trease and Evans, 1989)

Table 4 : Table showing % yield & colour of extracts of *Musa sapientum*

%Yield and Colour of several extracts of powdered Leaves of *Musa sapientum*

Extract obtained using the solvent	Colour of the Extract	Yield of the extract (% w/w)
Petroleum Ether	Dark green	4.26
Benzene	Yellowish brown	13.45
Chloroform	Yellowish green	19.85
Acetone	Green	14.36
Ethanol	Dark brownish green	41.60

Table 5: Table showing various tests of *Musa sapientum***Phytochemical Screening of several extracts of *Musa sapientum* Leaves**

S. N.	Test	Pet. Ether Extract	Benzene Extract	Chloroform Extract	Acetone Extract	Ethanol Extract
1	Carbohydrate	+	-	+	+	+
2	Glycoside	-	-	+	+	+
3	Fixed oil and fat	+	+	+	-	-
4	Protein & Amino acid	-	-	-	-	+
5	Terpenoid	+	+	-	+	+
6	Phytosterol	+	-	+	-	+
7	Alkaloid	-	-	-	-	-
8	Flavonoid	-	+	+	-	+
9	Saponin	-	+	+	-	+
10	Gum and mucilages	-	-	-	-	-

Where: (+) - Presence of constituents and (-) - Absence of constituents

The preliminary screening of phytochemicals (of *Musa sapientum* leaves extracts) found that Petroleum ether extract contains carbohydrates, fixed oils and fats, terpenoids and phytosterols, Benzene extract contains fixed oils and fats, terpenoids, flavonoids and saponins, Chloroform extract contains carbohydrates, glycosides, fixed oils and fat, phytosterols, flavonoids and saponins, Acetone extract contains carbohydrates, glycosides and terpenoids, Ethanol extract contains carbohydrates, glycosides, proteins and amino acids, terpenoids, phytosterols, flavonoids and saponins. On the basis of literature survey, found that flavonoids are responsible for the antiulcer activity, so we have concentrated our research work on two extracts only (Chloroform and Ethanol) because both contain flavonoids.

4.1.17 Thin Layer Chromatography study of different extracts

Thin layer chromatography (TLC) is a technique for separating dissolved chemical substances by virtue of their differential migration over glass plates or plastic sheets coated with a thin layer of a finely ground adsorbent, such as silica gel or alumina, that is mixed with a binder such as starch or plaster of Paris (Lala, P. K., 1981).

Preparation of plates

Slurry is prepared using Silica gel G for TLC with distilled water and coated on glass plates of 20cm x 5cm size with the help of conventional spreader, to a layer thickness of 0.25mm. The plates are first air dried and activated at 110°C for 30 minutes. After cooling the plates are kept in desiccator for future use.

Development of Chromatogram

Samples of ethanol and chloroform extracts were spotted on silica gel G coated plates with the help of capillary tube and in closed chambers the chromatograms were developed using different solvent systems at an angle of 70° at room temperature (around 30°C).

In each case the solvent system was allowed to run to a distance of 10 cm from the base line of application of the extracts on the plates. The time required for development of chromatogram varied from 30 to 45 minutes. After completion of the run, the plates were removed from the closed chamber and dried in air. These plates were viewed under U.V light before and after spraying of several spray reagents.

In all cases, the plates were heated at 105°C for 5-10 minutes after spraying. The colors of the spots developed and their R_f values were inspected in ordinary daylight as well as under long wave U.V light.

The results of the TLC studies have been furnished in the Table No.9-Table No.10. 5.5.3

Development of TLC plates

Silica gel G for TLC (Procured from Sisco Research Lab. Pvt. Ltd., Mumbai) was used for the preparation of the TLC plates.

Slurry was prepared with distilled water and coated on glass plates of 20cm x 5cm size with the help of conventional spreader, to a layer thickness of 0.25 mm.

The plates were first air dried, then activated at the temperature of 110° C for 30 minutes. The plates were allowed to cool then and kept in desiccators for future use.

With a pencil, two small notches were etched to the adsorbent layer from about 2cm of the bottom conventional spreader, to a layer thickness of 0.25 mm.

The plates were first air dried, then activated at the temperature of 110° C for 30 minutes. The plates were allowed to cool then and kept in desiccators for future use.

With a pencil, two small notches were etched to the adsorbent layer from about 2cm of the bottom of the plate. The notches on the edges of the plate, and each notch were in the same distance up from the bottom of the plate.

The base line must be little bit above from the solvent level in the chamber.

Spotting a sample of different extracts

Samples of aqueous and non-aqueous extracts were spotted on silica gel G coated plates with the help of capillary tubes. The narrow tip of the drawn-out capillary tube was used to touch the liquid extract sample and drawn up about 5 mm of the 30 solution.

The samples were spotted carefully without disturbing the surface of the adsorbent and the spots were about 3-3.5mm in diameter.

Developing the TLC plates

After spotting the samples on TLC plates, the plates were placed at an angle of 70° into the development chamber for the development of chromatogram, using different solvent systems at the room temperature.

The development chamber was left covered with the lid tightly. When the solvent front approached the top of the plate (10cm distance) the plates were removed from the chambers. The time required for development of chromatograms varied from 30-45minutes.

The solvent fronts were traced quickly with a pencil and dried in air.

Identifying the spots

These plates were viewed under the UV lamp before and after exposed to iodine vapour or concentrated H₂SO₄. In both cases, the plates were heated at 105°C for ten minutes. The colours of spots developed and their R_f values were inspected in daylight as well as long wave ultra-violet light (UV, 365nm). The results of the TLC studies have been reported below.



Ethanol: Ethyl Acetate: Water

(80:12:8)

Fig.7: TLC of ethanol extract of *Musa sapientum*- 6 spots



Ethyl Acetate: Formic Acid: Water: Gla.Acetic Acid

(85:5:8.5:1.5)

Fig.8: TLC of chloroform extract of *Musa sapientum*- 6 spots

Figure 8 & 9 : Image showing TLC of Ethanol & chloroform extract of *Musa sapientum*

4.1.18 Isolation and characterization of compounds by IR, NMR and Mass from ethanol extract of *Musa sapientum*

Isolation by fractionation method from ethanol extract of *Musa sapientum*

Take ethanol extract of *Musa sapientum* and then added petroleum ether to ethanol extract and shaking vigorously in separating funnel. The petroleum ether soluble portion discarded and the insoluble portion extracted with 80% ethanol. The aqueous ethanol extracts with ethyl acetate and then ethyl acetate layer shows aglycon flavonoids (compound A).

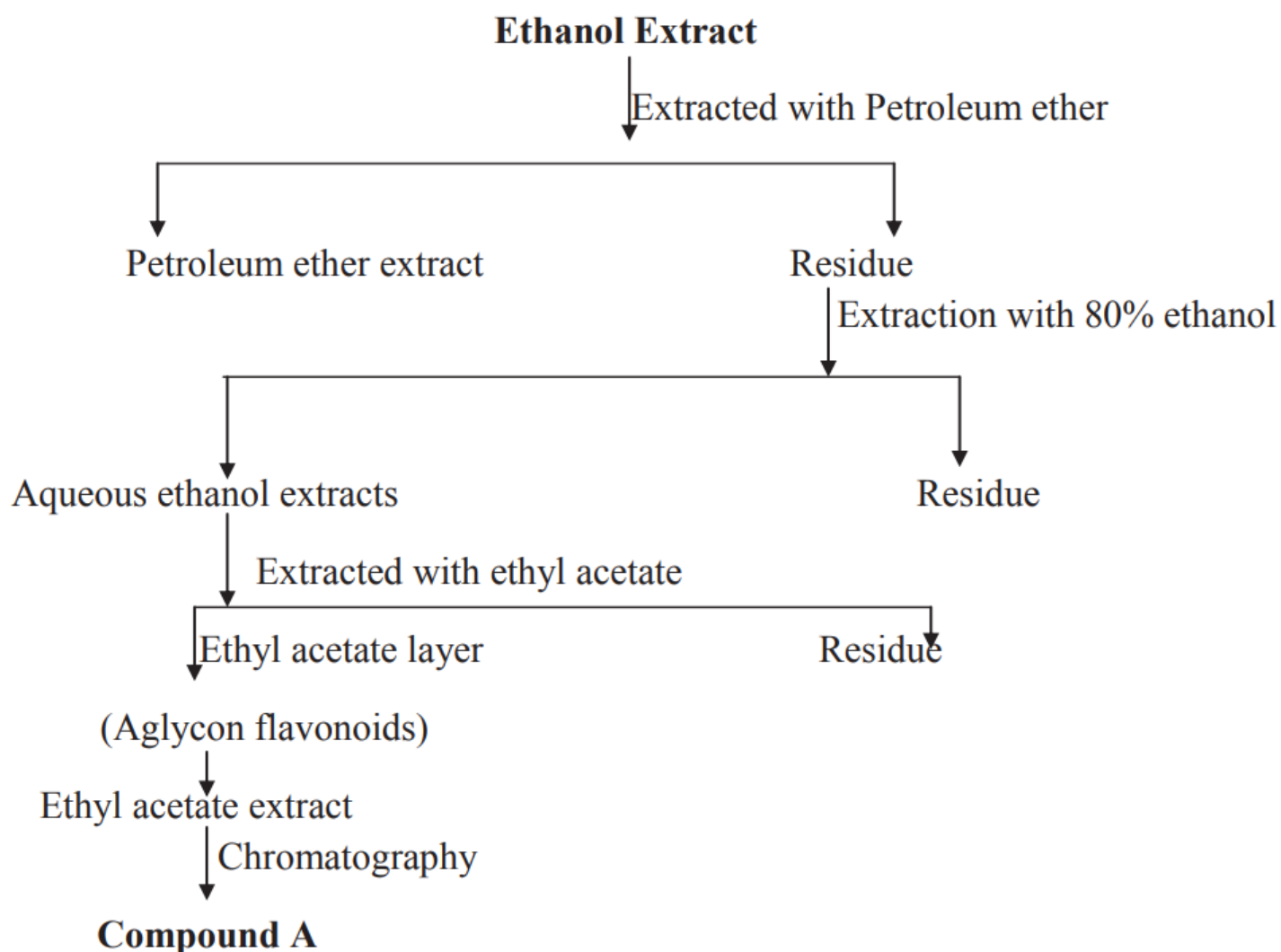


Figure 10 : Scheme for fractionation method of ethanol extract of *Musa sapientum*

4.1.19 Characterization of isolated compound A by IR, NMR and Mass from ethanol extract of *Musa sapientum*

IR Spectra of Isolated compound A from ethanol extract of *Musa sapientum* (Sharma, Y.R., 200710)

In IR spectra (Graph No. 3) of compound A,

Sharp peak is observed at:

781.6 cm⁻¹ presence of C-H Bending of Aromatic protons (Below 900 cm⁻¹)

1034.4 cm⁻¹ - presence of C=C-O-C (C-O Str)

(1020-1070 cm⁻¹) 1667.3 cm⁻¹ - presence of C=C Str -nonconjugated diene/conjugated diene, aromatics

(1450-1675 cm⁻¹) & C=O Str carbonyl group

(1630- 1680 cm⁻¹) 2919.7 cm⁻¹ - presence of C-H Str-alkanes

(2850-2960 cm⁻¹) 3142.9 cm⁻¹ - presence of C-H Str aromatics

(3020-3150 cm⁻¹) 3423.1 cm⁻¹ - Presence of O-H Str alcohols/phenols (H bonding)

4.1.20 MASS Spectra (Sharma, Y.R., 2007)

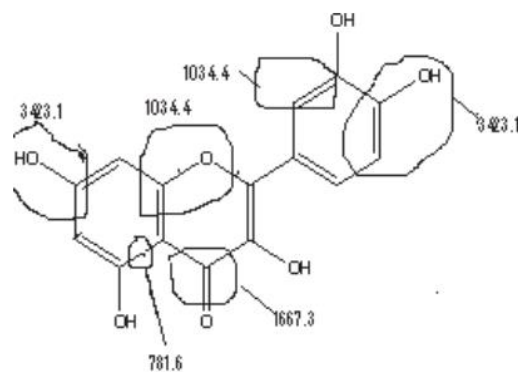
Mass spectrophotometer gives a record of relative abundance of ions according to the m/e ratio.

A peak corresponding to the ion of maximum abundance is called base peak.

The molecular ion or the parent ion peak may or may not be the base peak. M+1 and M+2 peaks also appear in very low abundance and are called isotope peaks.

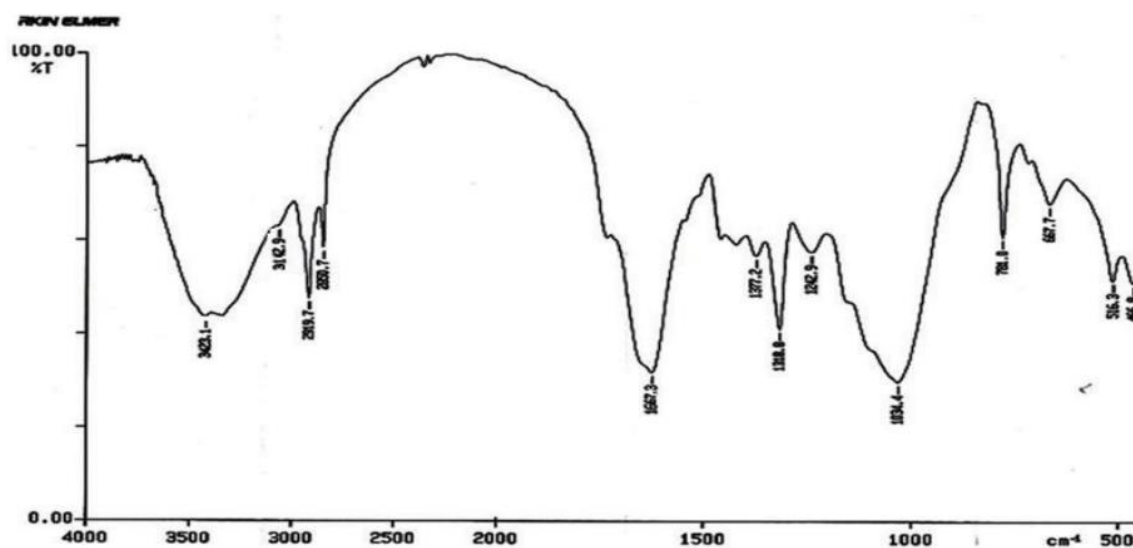
The fragmentations of molecular ion produce daughter ions of definite m/e value which help in structure determination.

In Mass spectra (Graph No.5) we get a molecular ion peak m/z 301.1(M+1) that is 301.1+1=302.1 matches molecular structure of the compound A (Fig.4.10). So the expected structure (Fig.4.11) can be shown as per the above findings:



Compound A

(Fig.1)



Graph No.3: IR Spectra of compound A (EEMS)

Figure 11 : Image showing molecular structure of compound A & mass spectra.

5.1 RESULT & DISCUSSION

Phoenix dactylifera

Macroscopic and microscopic evaluations, and extractive value in different medium and some colour characteristics of the powdered leaves of *Phoenix dactylifera* Linn. (Family: *Arecaceae*) are shown in above table and figures.

The characteristics help in the authentication and identification of the leaves of *Phoenix dactylifera* for material procured locally.

Musa sapientum

The chemical evaluation as well as chromatographic separation and evaluation mentioned in above table & figures reveal the presence of lots of chemical compounds in the leaves of *Musa sapientum*. Ethanol extract shows maximum yield (41.60% w/w) and petroleum ether shows minimum yield (4.26% w/w), whereas many a number of chemical compounds/constituents present in ethanol and in Chloroform extracts.

After the fractionation method of ethanol extract of *Musa sapientum* (EEMS) and through the various spectral interpretations, it has been found that compound A is Quercetin (Flavonoids) and similarly, in case of chloroform extract of *Musa sapientum* (CEMS), after fractionation method and through the various spectral interpretations it has been found that compound B is Rutin (Flavonoids).

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