JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

PHYTOPHARMACOLOGICAL EVALUATION OF MUSA SAPIENTUM FOR ULCER PROTECTIVE ACTIVITY

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ABSTRACT: The aim of present research work is phytopharmacological Screening of Musa sapientum for anti-ulcer activity. The Indomethacin-induced ulcer model was employed for screening anti-ulcerogenic activity because the model shows cytoprotection and gastric acid secretion activities**.** Indomethacin a nonsteroidal anti-inflammatory drug (NSAID), induced gastroduodenal ulceration via its ability to suppress prostaglandin synthesis**.** Groups of adult albino wistar rats were taken for the study. Rats were divided into groups each containing 6 animals. Group first is normal group received the saline for 7 days. Group second is indomethacin inducer group (20 mg/kg bw). The pH of gastric juice was observed as 3.846 of Musa sapientum (400 mg/kg bw) treated group and it showed the reduction in acidic pH (400 mg/kg bw) showed 3.327 pH. The free acidity was observed as mE/L of Musa sapientum (400 mg/kg bw) treated group 16.286 and it showed the reduction in acidity as compared to showed 16.948 mE/L.

KEYWORDS: Herbal, Extraction, Medicine, Authentication, Estimation, Screening

INTRODUCTION:

The drugs deduced from herbal source constitute the major part in most of the conventional system of medication. Plants are easily accessible, inexpensive and have fitted the immediate personnel needs as compared to other agents hence plants are employed for medication from era immemorial. Plant grounded drugs (natural drugs) may be used instantly, that is they may be gathered, dried and used as curative agents (crude drugs) or their chief constituents /active principles separated by various chemical process which are utilized as medicine (eg: digoxin isolated from Digitalis Purpurea is used in congestive cardiac failure) [(2).]

Peptic ulcer forms in the stomach or upper part of the small intestine and is the most frequent upper gastrointestinal acid-related disease of the digestive system, significantly affecting millions of people world wide. Peptic ulcers frequently occur along the lesser curvature of the antral end of the stomach or, more

rarely, in the lower end of the esophagus where stomach juices frequently reflux [3]. Gastric ulcer is predominantly characterized by damage to the gastric mucosa inthe stomach lining, resulting in abdominal pain, possible bleeding, chest pain, fatigue, vomiting and weight loss and other gastrointestinal symptoms [4and5].

The annual incidence rates of peptic ulcer diseases for the last decades were 0.1-0.19% for physician diagnosis [6]. Based on 31 published papers, in the last 3 decades that had reported incidence rate estimates for pepticulcer in the general population, it was found that the incidence rate was 1 case per 1000 person-years [7]. In a study from Sweden in which the symptomatic and asymptomatic peptic ulcer disease was considered the prevalence of peptic ulcer was 4.1%, where 19.5% of peptic ulcer disease were asymptomatic [8]. Every year, 4 million people are diagnosed with gastric ulcer disease around the world [9]. Complications are encountered in10%- 20% of these patients and 2%-14% of the ulcers will perforate [10]. An estimated 6000 people die every year because of the complications associated with stomach ulcer. 40, 000 people under go surgery in order to get relief from the persistent symptoms of ulcer annually. An estimated 15,000 deaths occur as consequence of peptic ulcer disease [11].

The multi factorial etiology of gastric ulcer includes bacterial infection, excessive alcohol intake, emotional stress, free radicals, the use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs), and nutritional deficiencies that disrupt the gastric mucosal barrier and make it vulnerable to normal gastric secretions. Ulcerogenic risk factors, such as excessive alcohol consumption and use of NSAID drugs, cause dispersal of the protective mucus gel and the phospholipid bilayer, resulting in acid back diffusion and mucosal injury secretions [12].The pathophysiology of ulcer is due mainly to an imbalance between aggressive factors (acid, pepsin, *H. pylori*, NSAIDs and local mucosal defensive factors (mucus, blood flow, endogenous prostaglandins(PGs) secretion, nitric oxide (NO),antioxidant and bicarbonate etc).The integrity of gastro-duodenal mucosa is maintained through a homeostatic balance between these aggressive and defensive factors [13]. The gastric and duodenal mucosa is covered by mucus and bicarbonate in order to protect against gastric acid. Mucus and bicarbonate are secreted by gastric epithelium and by Brunner's glands in the duodenum. The epithelium also has a role in acid protection. The apical cell membranes and the tight junctional complexes between the surface cells limit the penetration of hydrogen ion into the mucosa. In addition, mucosal blood flow transports nutrients and oxygen and bicarbonate to the surface to neutralise acid [14].

MATERIALSANDMETHODS

List of reagent and chemicals used

Table1: List of reagent and chemicals

S.No.	Reagents and chemicals	Company name
1.	Glacial Acetic Acid	Clorofilt ind
2.	Petroleum ether	Clorofilt ind
3.	Conc. H_2SO_4	Clorofilt ind.
4.	Ethanol	Clorofilt ind
5.	Nitroprusside	Fisher scientific
6.	Sodium Hydroxide	Merk
7.	Ammonia	Merk
8.	95% Alcohol	Clorofilt ind.
9.	Conc. HCl	Clorofilt ind.
10.	Magnesium	Himedia
11.	Chloroform	Clorofilt ind.
12.	1% Copper Sulphate Solution	Rankeem

Table2: List of glassware's

S.No.	Glassware	Company Name
1.	Beakers	Borosilicate
2.	Glass rod	Borosilicate
3.	Volumetric flask	Borosilicate
4.	Graduated pipette	Borosilicate
5.	Test tubes	Borosilicate

Table 3 List of Instruments

S.No	Instruments	Model
1.	Boiling water bath	Universal
2.	Hot Air Oven	Universal
3.	Weighing Machine	Sirtech digital weighing balance
4.	UV-Visible Spectrophotometer	Systronic(2202)
5.	Vortex shaker	Scientech(SE-146)

PLANTCOLLECTION

The medicinal plant Musa sapientum was collected locally from Bhopal, M.P. After cleaning, plant parts were dried under shade at room temperature for 3 days and then in oven at 45°C till complete dryness. Dried plant parts were stored in air tight glass containers in dry and cool place to avoid contamination and deterioration. Authentication of selected traditional plant – The medicinal plant Musa sapientum was authenticated by a plant taxonomist in order to confirm its identity and purity.

EXTRACTIONMETHOD

Generally three methods are employed in the extraction of plant materials. (1) Maceration, (2) Percolation (3) Successive Soxhlet Extraction. Maceration and percolation may be employed in extraction of thermo labile constituents. Soxhlet extraction is rapid and continuous and may be employed in extraction, which cannot be done by either maceration or percolation method. Due to the various advantages offered by soxhlet extraction, this method was selected for present study**.**

Solvent extraction of plant extract

❖ **Preparation of the extract-**

Coarsely powered plant parts of Musa sapientum was then extracted by successive extraction using different

organic solvents, defatted with petroleum ether (40-60^{0}C) and successively extracted with methanol for 36 hrs using soxhlet apparatus.[(1).]

Figure2: Soxhlet apparatus

Experimental work

Animals Protocol

IAECApproval All animal experiments were approved by Institutional Animal Ethics Committee (IAEC).

Animal used Weight

200±50gm **Strain** Wistar rat

Housing Condition- Animals were housed in a group of six inseparate cages under controlled conditions of temperature (22 ± 2°C). All animals were given standard diet (golden feed, New Delhi) and water regularly.

Induction of ulcer in rats:

Male Wistar rats weighing 200±50 were fasted for 24 hr with free access to water and rats randomly divided into groups.The control group received a vehicle (distilled water, 5ml/kg, p.o.) and Second group is inducer group which was treated only Indomethacin 20 mg/kg bw. And treatment groups III and IV were given Indomethacin 20 mg/kg bw and test sample (Extract- 400 mg/kg body weight) respectively 60 min prior to the Indomethacin (20 mg//kg, p. o.) for seven days. Standard group (V) was treated with the standard antiulcer drug (omeprazole 20 mg/kg, p.o,). The rats were sacrificed after one hours of Indomethacin administration and the stomach was removed and opened along the greater curvature.

Experimental design

Rats (n=30) were and optimized into following groups:

Group 1- Normal control

Group2-Inducer group Indomethacin 20mg/kg bw

Group3-Treated with Musa sapientum extract 400mg/kg bw

Group4-Treated with standard drug (Omeprazole) 20mg/kg bw

Parameters assessed for anti-ulcer activity

- Determination of Ulcer Index, Determination of pH and Volume of gastric juice and Free acidity determination

Ulcer index

The ulcer Index and percentage of ulcer inhibition were determined as follows:

$$\text{Ulcer index (UI)} = UN + US + UP \times 10^{-1}$$

Where,UN=Average number of ulcers per animal, US = Average of severity score,
UP=Percentage of animals with ulcers

Volume of gastric juice

The volume of gastric juice of each animal was measured after centrifugation with 1000rpm for 10 minutes and analyzed.

pH of gastric juice

A pH meter is used for determining pH after diluting 1ml of gastric juice aliquot with 1ml of distilled water.

Determination of free acidity

The free acidity was calculated by the formula:

$$\%yield = \frac{\text{Actual yield}}{\text{Theoretical yield}} \text{x}100$$

RESULT AND DISCUSSION:

PercentageYield

The yield of extracts received from the Musa sapientum is shown in Table: 4

Table4–Percentage Yield of crude extracts of Musa sapientum extract

S.no	Plantname	Solvent	Theoretical Weight	Yield(gm)	%yield
1	*Musa sapientum*	Petether	300	1.30	0.43%
2		Methanol	367	5.95	1.62%

PRELIMINARY PHYTOCHEMICAL STUDY:

Table5:Phytochemical testing of Musasapientum

S.No.	Experiment	Presence or absence of phytochemical test	
		Pet. Ether extract	Methanolic extract
1.	**Alkaloids**		
1.1	Dragendroff'stest	Absent	Present
1.2	Mayer'sreagent test	Absent	Present
1.3	Wagner'sreagent test	Absent	Present
1.3	Hager'sreagent test	Absent	Present
2.	**Glycoside**		
2.1	Borntrager test	Absent	Present
2.2	Legal's test	Absent	Present
2.3	Killer-Killiani test	Absent	Present
3.	**Carbohydrates**		
3.1	Molish's test	Absent	Absent
3.2	Fehling's test	Absent	Absent
3.3	Benedict's test	Absent	Absent
3.4	Barfoed's test	Absent	Absent
4.	**Proteins and Amino Acids**		

4.1	Biuret test	Absent	Present
5.	**Flavonoids**		
5.1	Alkaline reagent test	Absent	Present
5.2	Lead Acetate test	Absent	Present
6.	**Tannin and Phenolic Compounds**		
6.1	Ferric Chloride test	Absent	Present
7.	**Saponin**		
7.1	Foam test	Absent	Present
8.	**Test for Triterpenoids and Steroids**		
8.1	Salkowski's test	Absent	Absent
8.2	Libbermann-Burchard's test	Absent	Absent

QuantitativeAnalysis

Preliminary phytochemical testing of crude extracts confirmed the presence of phenolics and flavonoids in plant material. To estimate their amount total phenolic (TPC) and total flavonoid content (TFC) assays were performed.

TOTAL PHENOLIC CONTENT (TPC) ESTIMATION:

Table7 Standard table for Gallic acid

S.No.	Concentration(µg/ml)	Absorbance
1.	20	0.136
2.	40	0.169
3.	60	0.193
4.	80	0.230
5.	100	0.264

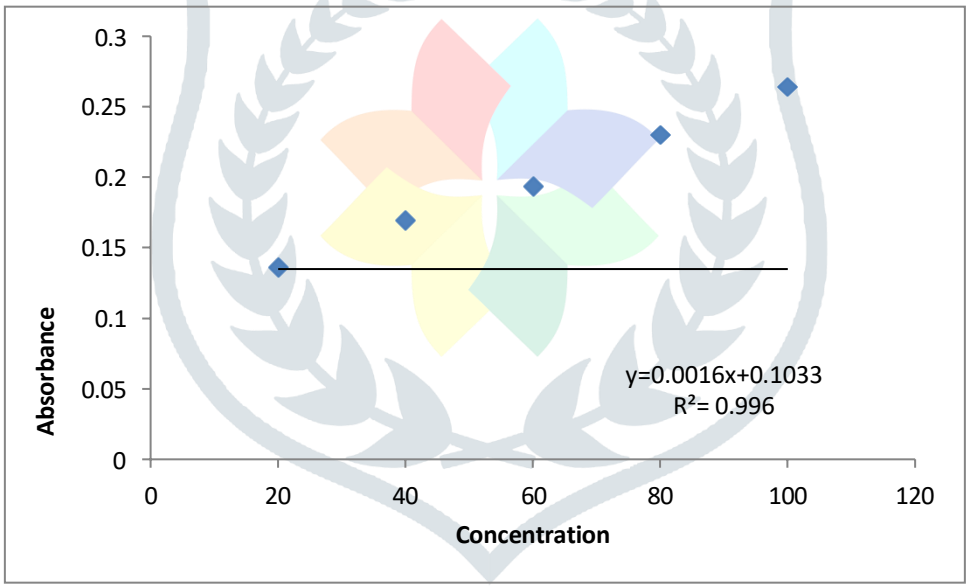

Figure3:Graph represent standard curve of Gallic acid

TOTAL PHENOLIC CONTENT IN EXTRACT:

Table 8 Total Phenolic Content in extracts

	Total phenolic content (mg/gm equivalent to gallic acid)
Extracts	***Musa sapientum***
Absorbance (mean±SD)	0.196±0.09
TPC	93

TOTAL FLAVONOIDS CONTENT (TFC) ESTIMATION:

Table 9 Standard table for Rutin

S. No.	Concentration (µg/ml)	Absorbance
1.	20	0.155
2.	40	0.170
3.	60	0.189
4.	80	0.201
5.	100	0.239

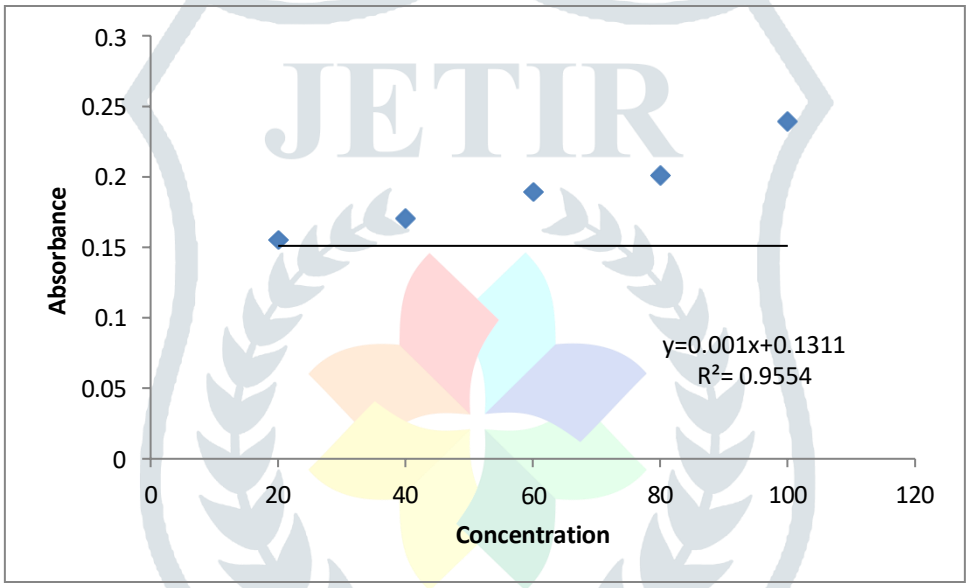

Figure4:Graph represent standard curve of Gallic acid

Total Flavonoid Content in extract

Table 10 Total Flavonoid Content in extracts

	Total flavonoid content (mg/gm equivalent to rutin)
Extracts	***Musa sapientum***
Absorbance(mean±SD)	0.170±0.009
TFC	39

Invitro Antioxidant Assays

Table11-DPPH radical scavenging activity of Std. Ascorbic acid

Concentration(µg/ml)	Absorbance	% Inhibition
20	0.459	50.59203
40	0.411	55.75888
60	0.314	66.20022
80	0.258	72.2282
100	0.139	85.03767
Control	0.929	
IC50		22.59

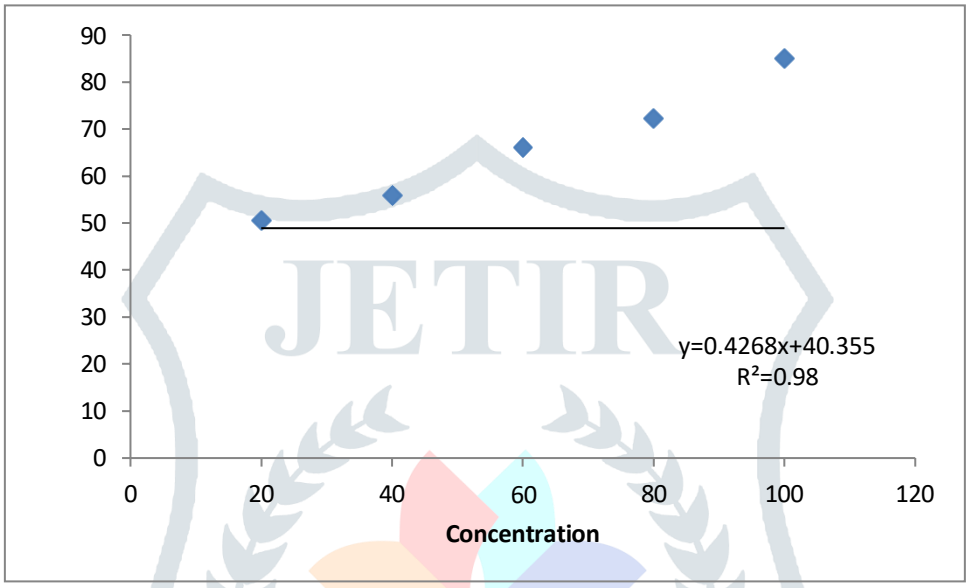

Figure 5: DPPH radical scavenging activity of Std. Ascorbic acid

DETERMINATION OF ULCER INDEX

TABLE 14: Observation of Ulcer Index

Groups	Ulcer index
	Mean
Group I-Normal Control	0
Group II Musa sapientum Extract treated (400mg/kg) group	2.599±0.260
Group III Standard (Omeprazole20mg/kg bw)	1.770±0.499

DETERMINATIONOFVOLUMEOFGASTRICJUICE:

Table15:Observation of volume of gastricjuice

Treatment Group	Volume of gastric juice
Group I-Normal Control (Saline)	1.646± 0.448
Group II Inducer Indomethacin 20 mg/kg bw)	5.573± 0.886
Group III–(Musa sapientum Extract treated 400mg/kg)	2.363± 1.009
Group IV-Standard (Omeprazole 20mg/kg bw)	1.854± 0.750

DETERMINATION OF PH OF GASTRIC JUICE:

Table16: Observation of pH of gastricjuice

Treatment Group	pH of gastric juice
Group I-Normal Control (Saline)	4.187±0.776
Group II inducer Indomethacin 20mg/kgbw)	2.475±0.915
Group III–(Musa sapientum Extract Treated 400mg/kg)	3.846±1.019
Group IV-Standard (Omeprazole 20mg /kg bw)	4.137±0.783

Free acidity determination:

Table17:Observation of free acidity in indomethacin induced peptic ulcer in rats:

Treatment Group	Free acidity determination (mE/L)
Group I-Normal Control (Saline)	15.257±3.544
Group II inducer Indomethacin 20mg/kg bw)	24.375±2.562
Group III– (Musa sapientum Extract treated 400mg/kg)	16.286±1.471

Group IV-Standard (Omeprazole20mg/kg bw)	16.645±1.755

Figure8:**Normal stomach of rat**

Figure 9:**Ulcer is induced in rat stomach**

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6. DISCUSSION

The Indomethacin-induced ulcer model was employed for screening anti-ulcerogenic activity because the model shows cytoprotection and gastric acid secretion activities**.** IND, a nonsteroidal anti-inflammatory drug (NSAID), induced gastro duodenal ulceration via its ability to suppress prostaglandin synthesis**.**

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Four groups of adult albino wistar rats were taken for the study. Rats were divided into groups each containing 6 animals. Group first is normal group received the saline for 7 days. Group second is indomethacin inducer group (20 mg/kg bw). Third group is Musa sapientum extract (400 mg/kg bw) and Group fourth is standard (Omeprazole 20 mg/kg bw). The extract of Musa sapientum was evaluated by using indomethacin induced peptic ulcer model. There was a significant measured gastric ulcer index in the stomach of Musea sapientum (400 mg/kg bw) treated animals. The volume of gastric juice was observed as 2.363 ml of Musa sapientum (400mg/kg bw). The pH of gastric juice was observed as 3.846 of Musa sapientum (400 mg/kg bw) treated group. The free acidity was observed as mE/L of Musa sapientum (400 mg/kg bw) treated group 16.286.

SUMMARY AND CONCLUSION:

The plant sample kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders.

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