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RESEARCH ARTICLE

A PHARMACOLOGICAL STUDY AND EVALUATION 0F ANTIOXIDANT ACTIVITY OF MERREMIA EMARGINATA IN WISTAR RATS

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ABSTRACT

Merremia emarginata whole plant was subjected to preliminary phytochemical investigation and was found that it possess alkaloids, steroids, glycosides, flavonoids, tannins, carbohydrates and proteins. The extracts prepared by using polar solvents have demonstrated the dose dependent Antioxidant activity. The Methanolic extract of Merremia emarginata whole plant has shown Antioxidant activity in various screening models .Our study has justified the claim of native herbal practitioners that the plant extract has antioxidant activity and Superoxide anion scavenging activity useful in treating gastric disorders.

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INTRODUCTION

Ailments like gastritis, peptic ulcer, hepatic disorder and metabolic disorders (diabetes) are appearing as a major threat in the future. These diseases are major concern in front of researcher because contributing factors are increasing in developing world. The present scenario, the lifestyle doesn't permits us to have healthy food habits this put us in more vulnerable situation to have various gastric ailments. This leads to search of novel drug particularly of natural origin. Gastropthy is associated to the injury caused to gastric mucosa and damaging the epithelial cells. Gastritis is inflammation of gastric mucosa. Hence author's aim is to evaluate the antioxidant activity, gastro protective activity along with hepatoprotective and anti diabetic activities. The liver is an important organ as it regulates many imperative metabolic functions. Hepatic damage is associated with alteration of these metabolic functions. Liver is the first organ in the body which expose to toxins absorbed from the GIT resulting in many liver diseases, this is key organ of metabolism and excretion. Thus liver diseases remain one of the serious health concerns.

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A common metabolic disorder known as Diabetes mellitus with micro-and macrovascular complications those results in significant morbidity and mortality. It is considered as one of the cause among five leading causes of death in the world. Diabetes is in the top five of the most significant diseases in the developed world and is still gaining significance. Since the existing drugs for the above said disorders encounter many side effects and need for prolonged treatment including questionable efficacy in the treatment, these reasons force the area of research to find improved treatments which will counteract the side effects and drawbacks of the existing treatment. Herbal drugs are having diversified uses are always an alternative option to the synthetic drugs which are well known for their side and adverse effects. Hence under these conditions exploring new cures from plants source will always be beneficial because of less side effects. On the above facts the objective of this study to evaluate Merremia emerginata as a possible cure to above said disorders.

Merremia emerginata

Merremia emerginata belong to the family *Convolvulaceae,* is a flowering creeping perennial herb grows throuout India, China and Nepal. The Merremia emerginata is found in planes and at an altitude of about 900-1000 meters.

In Ayurveda, the roots & leaves of Merremia emerginata are used to treat ailments like inflammation, flatulance, diuresis, paralysis, etc, whole plant of Merremia emerginata is reported to contain alkaloids glycosides, phenolic & flavonoids along with carbohydrates and aminoacids. Much research has been undertaken to evaluate the drug to treat various ailments and to evaluate gastroprotective.

Plan of Work

- Preparation of Methanolic extracts of *Merremia emerginata* by using soxhlet extraction.
- Phytochemical analysis of Methanolic extracts of *Merremia emerginata*.
- To study the acute toxicity of the Methanolic extracts of *Merremia emerginata* by OECD 423 guidelines.

To evaluate antioxidant activity. The following in-vitro models were carried out.

- Reducing power.
- Superoxide anion scavenging activity.
- Hydroxyl radical.

MATERIALS AND METHODS

Collection of Plant Material and authentication: The *Merremia emarginata* whole plant was collected from Bidar forest area. A herbarium specimen is deposited in our college museum identification and authentication was done by Dr Malikarjun Patil of pharmacognosy department of Karnataka college of pharmacy Bidar. The powder obtained was subjected to successive soxhelt extraction with the solvents with increasing polarity i.e. petroleum ether, chloroform, methanol and water.

Preparation of methanolic extracts of M emarginata: The authenticated whole plant of *Merremia emarginata* were dried in shade and powdered coarsely. Extraction was done according to standard procedure using analytical grade solvents. The coarse powder of the leaves was Soxhlet extracted with the solvents with increasing order of polarity i.e. petroleum ether (60-80^{o}C), chloroform (59.5-61.5^{o}C), methanol (64.5-65.5^{o}C) ME1, and hydroalcoholic extract (methanol and water 50:50 ratio) ME2. After defating with petrolium ether, methanolic extract was also prepared. The extracts so obtained were concentrated under reduced pressure. In addition the shade-dried powder was extracted directly with methanolic (hydro-alcoholic) extract which was used for pharmacological investigations after subjecting it to preliminary qualitative photochemical studies. The extracts were concentrated under reduced pressure and stored in desiccators until further use and the percentage yield of corresponding extracts were calculated.

Antioxidant activity

In vitro anti-oxidant activity: The following in-vitro models were carried out to evaluate antioxidant activity.

- Reducing power.
- Superoxide anion scavenging activity.
- Hydroxyl radical.

Reducing power of *M emarginata* : This method is based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicate increase in the antioxidant activity.In this method substances which have reduction potential react with potassium ferriccyanide(Fe3+) to form potassium ferrocyanide (Fe2+).potassium ferrocyanide then reacts with ferric chloride to form ferric ferrous colored complex. which is measured at 700nm.Increase in absorbence of the reaction mixture indicate the reducing power of the sample.1.The reducing power of Methanolic extract (ME1) of whole plant of *Merremia emarginata* .

Procedure: Different doses of Methanolic extracts (ME1) of Merremia emarginata whole plant were mixed in 1 ml of distilled water so as to get 20µg, 40µg, 60µg, 80µg, and 100µg concentrations. This was mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50ºC for 20 minutes. A portion (2.5 ml) of Trichloroacetic acid (10%) was added to the mixture, which was then centrifused at 3000 rpm for 10 minutes.The upper layer of the solution (2.5 ml) was mixed distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance (OD) was measured at 700 nm.Increase in absorbance of the reaction mixture indicate increase in reducing power. The % reducing power was calculated by using the formula:

$$\% \text{ increase in absorbance} = \frac{\text{Test OD} - \text{Control OD}}{\text{Control OD}} \times 100$$

The results are compiled in Table No. 3 and graphically shown in Fig No. 2

Superoxide anion scavenging activity: Oxygen is essential for the survival of aerobic cells, but it has long been known to be toxic to them when supplied at concentration greater than those in normal air. Although super oxide anion is a week oxidant, it gives generation of powerful and dangerous hydroxyl radicals as well as single oxygen, both of which contribute to oxidative stress.Numerous biological reaction generate superoxide anions which are highly toxic species. The biochemical mechanisms responsible for oxygen toxicity include lipid peroxidation and the generation of H_2O_{2+} the superoxide radical, O_{2*}. This superoxide radical can inhibit or propagate the process of lipid peroxidation. Measurement of superoxide anion scavenging activity of *Merremia emarginata* whole plant was done by using the method explain as follows.

Procedure: Various concentrations of Methanolic extract of *Merremia emarginata* whole plant solutions were prepared such that each 0.1 ml contains 20, 40, 40, 60,80 and 100 µg. About 1 ml of Nitroblue tetrazolium (NBT) solution (156µM In 100 mM phosphate buffer, pH 7.4) and 1 ml of Nicotinamide adenine dinucleotide (NADH) solution (468 µM PMS in 100 mM phosphate buffer, pH (7.4) were mixed 0.1 ml of various concentrations of sample of Methanolic extract of *Merremia emarginata* whole plant and standard in water was mixed, The reaction was initiated by adding 10µl of Phenazine Methosulphate(PMS) solution (60µM PMS in 100 mM phosphate buffer,pH7.4)to the above mixture and was incubated at 25^{0} C for 5 minutes. The absorbance was at measured 560nm against blank. Decrease absorbance of the reaction mixture indicate superoxide anion scavenging activity,

$$\% \text{ increase in absorbance} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

Hydroxyl radical scavenging: In biochemical systems, superoxide radical and H_2O_2 react together to form the hydroxyl radical, OH, this can attack and destroy almost all known biochemical Hydrogen peroxide occurs naturally at low concentration level in environment and air. In the body, H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radical(OH).that can initiate lipid peroxidation and cause damage to DNA and erythrocytes. Phenyl hydrazine when added to erythrocyte hosts peroxidation of endogenous lipids and alteration of medicine fluidity. This peroxidation damage to erythrocytes is probably initiated by active oxygen species like O_{2*}, OH^* and H_2O_2 which are generated in solution from auti-oxidation of phenyl hydrazine. This forms the basis of the experiment.

Hydroxyl radical generation by phenyl hydrazine has been measured by the 2-deoxyribose degradation assay of Hathwell and Gutteride. In 50 mM phosphate buffer (pH7.4)1 mM deoxyribose, 0.2 mM phenyl hydrazinehydrochloride were prepared. 0.6 ml of 1 mM deoxyribose and 0.4 ml of Methanolic extracts of Merremia emarginata (varying doses 20, 40, 60, 80 and 100 µg) or sodium metabisulphate (25µg Std.) were mixed and phosphate buffer was added to make the volume to 1.6 ml.

The reaction mixture was incubated for 10 min and 0.4 ml of 0.2mM phenyl hydrazine HCl was added and incubated for 1 hr and 1 ml each of 2.8% TCA and 1% (w/v) of thiobarbituric acid were added. The reaction mixture was heated for 10 minutes on a boiling water bath. The tubes were cooled and absorbance was taken at 532 nm. The absorbance of the reaction mixture was inversely proportional to the hydroxyl radical scavenging activity. The % reduction in absorbance was calculated.

RESULTS

Successive Soxhlet extraction process has yielded 3.40% of dark green and sticky coloured petroleum ether extract 2.40% of dark greenish black coloured and sticky chloroform extract, 6.80% dark brown coloured methanolic extract and brown coloured hydroalcoholic 4.2 percent.

Results of phytochemical screening: It is observed from the phytochemical study that Alkaloids, Glycosides, Flavanoids, Tannins and Saponins are present in methanolic extract. Glycosides and steroids are present in chloroform and Petroleum ether extract. Results are compiled in Table No.02.

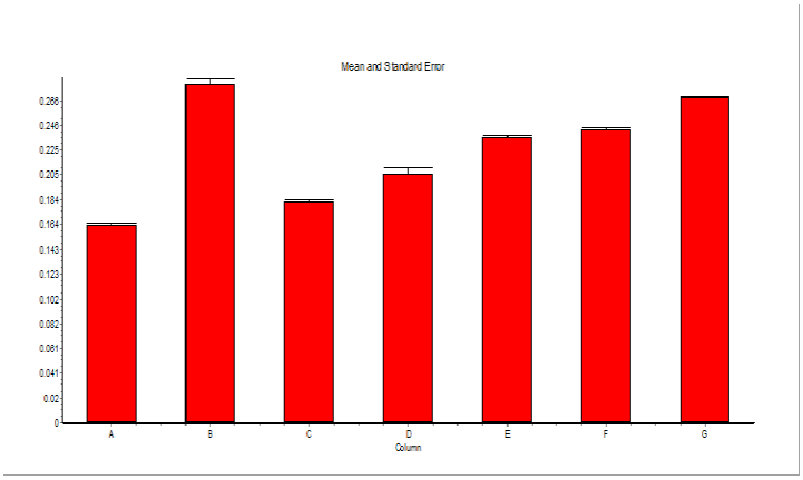
Table 1. Successive Soxhlet extraction Merremia emarginata

SL.NO	Solvents	Colour &consistency	% Yield
1	Pet.Ether	Dark green& sticky	3.40
2	Chloroform	Greenish black & sticky	2.40
3	Methanol (ME1)	Dark brown& sticky	6.80
4	Hydroalcoholic extract (ME2) Methanol, water in a ratio 50:50	Brown & sticky	4.2

Table 2. Preliminary phytochemical screening

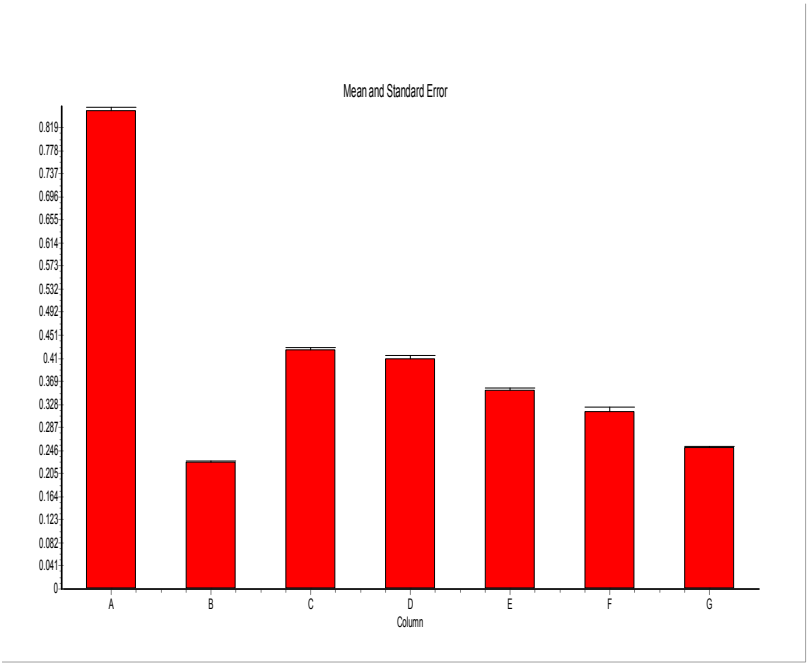
Constituents	Pet. Ether Extract.	$CHCL_3$Extract.	Methanolic extract.
Alkaloids	-	-	+
Carbohydrates	+	+	+
Glycosides	-	+	+
Steroids	+	-	-
Flavanoids	+	+	+
Saponins	-	-	-
Fixed oil And fats	-	-	-
Tannins	-	-	+
Protein & Amino acids	+	+	+
Mucilage	-	-	-

\+ = PRESENT.
\- = ABSENT



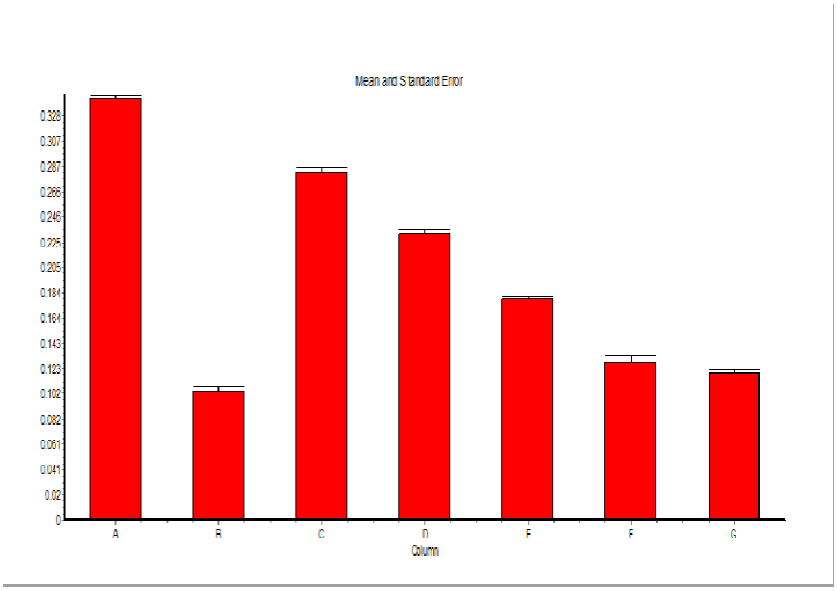
A = CONTROL GROUP B = STANDARD 25 µg C= METHANOLIC EXTRACT 20µg
D = METHANOLIC EXTRACT 40 µg E = METHANOLIC EXTRACT 60µ
F= METHANOLIC EXTRACT 80µg G = METHANOLIC EXTRACT 100µg

Fig. 1. Reducing power activity of Methanolic extract of Merremia emarginata.



A =CONTROL GROUP B= STANDARD 25 µg C = EXTRACT 20 µg D = EXTRACT 40µg E = EXTRACT 60 µg F= EXTRACT 80µg G= EXTRACT 100µg

Fig. 2. Superoxide anion scavenging activity of whole plant *Merremia emarginata*



A =CONTROL GROUP B= STANDARD 25 µg C = EXTRACT 20 µg D =EXTRACT 40 µg E =EXTRACT 60 µg F = EXTRACT 80µg G = EXTRACT 100 µg Hydroxyl radical scavenging activity of methanolic extract of Merremia emarginata. (AFTER 4 HR)

Fig. 3. Hydroxyl radical scavenging activity of methanolic extract of merremia emarginata: (AFTER 1 HR)

Conclusion

The *Merremia emarginata* whole plant contains alkaloids, steroids, glycosides, flavonoids, tannins, carbohydrates and proteins. Methanolic extract of *Merremia emarginata* whole plant increased the PH of Gastric juice. Methanolic extract of *Merremia emarginata* whole plant reduce total acidity and free acidity. Treatment with Methanolic extract of *Merremia emarginata* whole plant has significantly reduced the volume of gastric juice. Methanolic extract *Merremia emarginata* whole plant shows significant anti-oxidant activity in Vitro models.

Scope for further study

Since, our study has indicated only the usefulness of *Meeremia emarginata,* whole plant extract Shows Antioxidant activity, there is room for further study to identify, isolate, characterize and evaluate the active principle responsible for the Antioxidant activity of the plant. In addition toxicological aspects of the plant is not studied in this project work. Hence, a study may be undertaken from the toxicological point of view. Even formulation and evaluation of this herb may also be studied.

Summary

Merremia emarginata whole plant was subjected to preliminary phytochemical investigation and was found that it possess alkaloids, steroids, glycosides, flavonoids, tannins, carbohydrates and proteins. The Methanolic extract of Merremia emarginata whole plant has shown great potential in various screening models of anti-oxidant activity.

It has demonstrated the gastro protective /anti-ulcer activity which was evident by decrease in the ulcer index.Our study has justified the claim of native herbal practitioners that the plant extract is useful in treating the different Diseases and disorders.

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