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Invitro antioxidant and antiarthritis activity of extracts and fractions of *Merremia emarginata*

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ABSTRACT

Merremia emarginata Burm.f (convolvulaceae) is a perennial, much branched herb (creeper). It is found widely distributed all over the India, especially in damp places in upper gangetic plain, Gujarat, Bihar, West Bengal, Western- Ghats, ascending up to 900m in the hills, Goa, Karnataka in India, Ceylon and Tropical Africa. All the genus of the family are economically important. It was traditionally used in various inflammatory conditions such as Rheumatoid arthritis, Alkylosing spondylitis, Osteoarthritis, Gout etc and also in conditions such as Pain, Ulcer, Cancer and Wounds. The antinociceptive, anti-inflammatory and anti-oxidant activities of IP have already been scientifically proven. The present invitro anti-arthritic and antioxidant study of Merremia emarginata was undertaken to substantiate its folkloric uses in the treatment of arthritis. Merremia emarginata is also known as Ipomoea reniformis chois.

Keywords: *Merremia emarginata*, *Ipomoea reniformis*, Convolvulaceae, Antiarthritis, Antioxidant

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INTRODUCTION

The primary advantage of *in vitro* work is that it permits an enormous level of simplification of the system under study, so that the investigator can focus on a small number of components [1,2]. For example, the identity of proteins of the immune system (e.g. antibodies), and the mechanism by which they recognize and bind to foreign antigens would remain very obscure if not for the extensive use of *in vitro* work to isolate the proteins, identify the cells and genes that produce them, study the physical properties of their interaction with antigens, and identify how those interactions lead to cellular signals that activate other components of the immune system [3]. Cellular responses are species-specific, lending cross-species analysis problematic. Newer methods of same-species-targeted, multi-organ studies are available to bypass live, cross-species testing [4].

In recent years, the emerging concepts in biology systems and the translation of these concepts into clinical trials drive the developments of in vitro diagnostics applications. Upon the comprehensive and emerging clinical needs, the probes for in vitro diagnostics are needed to be efficiently produced, highly sensitive, quantitative, rapid, handy, and even multiplexed to detect and monitor the biomolecules (e.g., DNA, RNA, and proteins) or bioentities (e.g., cancer cells, bacteria, and virus) from small amount of diverse clinical samples (e.g., tissues, blood, serum, and urine). The development of simple, reliable, and sensitive probes is a strong current scientific priority [5,6].

MATERIALS AND METHODS

2.1 Plant material and Preparation of Extracts and Fractions

Fresh plant materials of *Merremia emarginata* Burm. F whole plant was collected during January & February 2010 from the surroundings of Tirunelveli, were botanically identified and authenticated by Prof. Jayaraman, Plant Anatomy Research Centre, Tambaram, Chennai, Tamilnadu, India.

The shade dried whole plant of *Merremia emarginata* were coarsely powdered and extracted with ethanol using soxhlet extraction apparatus until exhaustive extraction. The solvent was removed using rotary vacuum evaporator and solvent free extracts were subjected for column chromatography (silica gel 60-120) and further broad fractioned by successive solvents using hexane, chloroform, ethylacetate and methanol solvents. The different fractions collected were concentrated by rotary vacuum evaporator and subjected for *in vitro* antiarthritic, and antioxidant studies.

2.2 *In vitro* anti-arthritic activity by inhibition of protein denaturation method

Procedure

1. The Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0 .05ml of various extracts/fraction of both plants (250 µg/ml).
2. Test control solution (0.5ml) consist of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0 .05ml of distilled water.
3. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution (250 µg/ml).
4. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml 0f Diclofenac sodium (250 µg/ml). All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°C for 20 min and the temperature was increased to keep the samples at 57°C for 3 min. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416nm[7,8]

The percentage inhibition of protein denaturation can be calculated as,

Percentage inhibition = [100-(optical density of test solution – optical density of product control) ÷ (optical density of test control)] ×100.

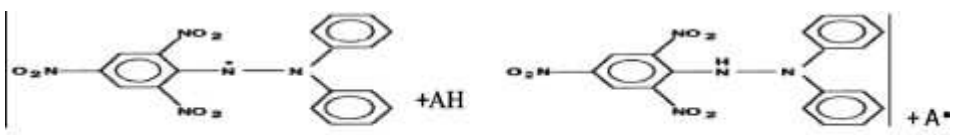
The control represents 100% protein denaturation .The results were compared with Diclofenac sodium (250 µg/ml). The percentage inhibition of protein denaturation of different concentration was tabulated.

2.3 *In vitro* antioxidant activity

Antioxidant activity should not be concluded based on a single antioxidant test model. And in practice several *in vitro* test procedures are carried out for evaluating antioxidant activities with the samples of interest. Another aspect is that antioxidant test models vary in different respects. Therefore, it is difficult to compare fully one method to other one. Generally *in vitro* antioxidant tests using free radical traps are relatively straight forward to perform. Among free radical scavenging methods, DPPH method is furthermore rapid, simple (i.e. not involved with many steps and reagents) and inexpensive in comparison to other test models. On the other hand ABTS decolorization assay is applicable for both hydrophilic and lipophilic antioxidants.

2.4 DPPH scavenging activity

The molecule 1, 1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. The delocalization of electron also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 517 nm. When a solution of DPPH is mixed with that of a substrate (AH) that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet color.



Diphenylpicrylhydrazyl (free radical) Diphenylpicrylhydrazine (nonradical)

In order to evaluate the antioxidant potential through free radical scavenging by the test samples, the change in optical density of DPPH radicals is monitored. The alcoholic extracts and their respective fractions of both plants in different concentration (0.2 ml) is diluted with methanol and 2 ml of DPPH solution (0.5 mM) is added. After 30 min, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation as given below:

$$\% \text{ inhibition of DPPH radical} = ([A_{br} - A_{ar}]/A_{br}) \times 100$$

where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place.

2.5 Hydrogen peroxide scavenging (H_2O_2) assay

Human beings are exposed to H_2O_2 indirectly via the environment nearly about 0.28 mg/kg/day with intake mostly from leaf crops. Hydrogen peroxide may enter into the human body through inhalation of vapor or mist and through eye or skin contact. H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($OH^{\bullet}$) that can initiate lipid peroxidation and cause DNA damage in the body. The ability of plant extracts to scavenge hydrogen peroxide can be estimated. A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. The alcoholic extracts and their respective fractions of both the plants in different concentrations is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

$$\% \text{ scavenged } (H_2O_2) = [(A_i - A_t)/A_i] \times 100$$

where A_i is the absorbance of control and A_t is the absorbance of test.

2.6 Scavenging of 2,2'-azino-bis (3-ethyl benzo thiazole-6-sulphuric acid) di ammonium salt (ABTS)radical cation

The working solution was prepared by mixing stock solutions of 7 mM ABTS and 2.4 mM potassium persulphate in equal amounts and allowing them to react for 12 h at room temperature in the dark. The resulting solution was later diluted with distilled water, and the absorbance read at 734 nm using a UV–visible spectrophotometer. A total of 1 ml of freshly prepared ABTS solution was added to 1 ml of the alcoholic extracts and their respective different fractions of different concentrations, the reaction mixture was vortexed for 10 s and the absorbance was measured at 734 nm after 6 min.

2.7 Scavenging of Hydroxy Radical in the Para-Nitraso Di methyl aniline (p-NDA)

To the solution containing ferric chloride (0.1mM, 0.5ml) ascorbic acid (0.1ml of 0.5ml) H_2O_2 (2mM of 0.5ml) pNDA (0.01mM of 0.5ml) in phosphate buffer (pH 7.44, 20mM) were added with the various concentrations of the extracts and their respective different fractions of the plants or standards in distilled DMSO (0.5ml) to produce a final volume of 3ml. Absorbance was measured at 440nm[14,15]

RESULTS

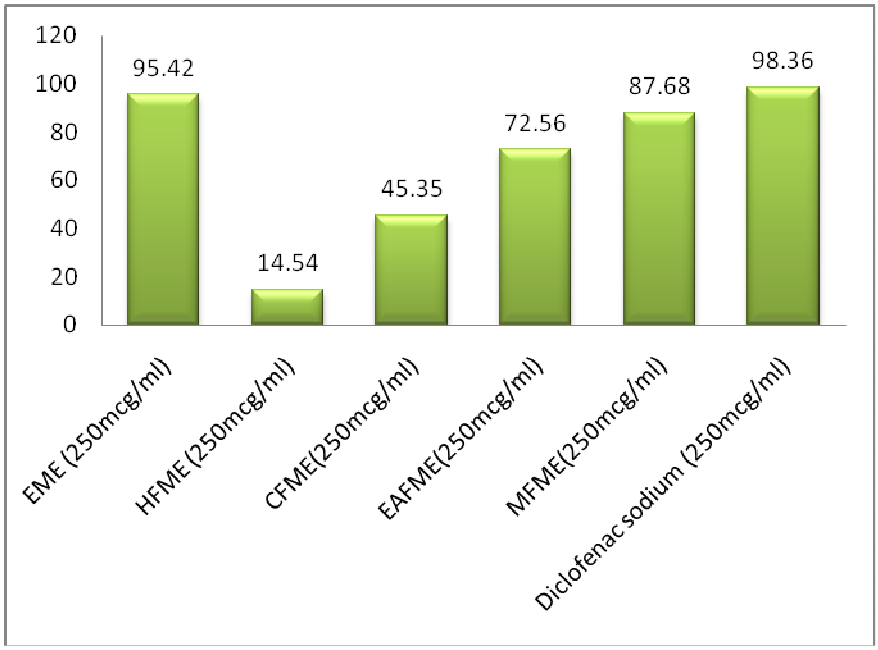
3.1 *In vitro* antiarthritis

Table 1 3.1 a) Effect of extracts and fractions of *Merremia emarginata* on inhibition of protein denaturation

S.No	Extracts/fractions/Standard(250µg/ml)		% percentage inhibition
1	Extract	EME	95.42
2	Fractions	HFME	14.54
3		CFME	45.35
4		EAFME	72.56
5		MFME	87.68
6	Standard	Diclofenac sodium	98.36

Concentrations of extracts/fractions were taken 250µg/ml for all the sample.

FIG 1 Effect of extracts and fractions of *Merremia emarginata* on inhibition of protein denaturation



3.2 *In vitro* antioxidant activity

Table 2 3.2 a) Effect of extracts and fractions of *Merremia emarginata* on DPPH, H_20_2, ABTS, p-NDA

S.No	Extracts/Fractions/ Standard		IC_{50} Values in(µg/ml) ±SEM			
			DPPH	H_2O_2	ABTS	$_P$-NDA
1	Extract	EME	26.5 ± 1.2	77.6 ± 1.45	14.6 ± 0.95	>100
2	Fractions	HFME	78.7 ± 0.98	>100	37.8 ± 0.47	>100
3		CFME	67.7 ± 0.34	>100	36.7 ± 1.23	>100
4		EAFME	45.7 ± 1.43	>100	23.8 ± 0.37	>100
5		MFME	27.5 ± 0.57	85.6 ± 1.13	18.6 ±1.78	>100
6	Standard	Ascorbic acid	12.3 ± 0.98	57.6 ± 0.57	18.5 ± 0.68	>100
7		Rutin	0.89 ± 0.07	1.25 ± 0.79	12.7 ± 1.18	-
8		Butylated hydroxyanisole BHA	-	18.57 ± 1.09	-	-

The IC_{50} values were determined 3 times, ±SEM, The potent antioxidant activity of extracts and fractions were determined by minimal IC_{50} with different model.

The preliminary *in vitro* screening was carried out to find out the active extracts and fractions of *Merremia emarginata* for further proceeding of biological studies.

The results of *in vitro* antiarthritis studies revealed that the percentage inhibition of protein denaturation was significantly exhibited by alcoholic extract of ME (95.42) and also ethyl acetate (72.56) and methanol fraction (87.68) of ME. The percentage inhibitions more than 70% were taken for biological studies.

The *in vitro* antioxidant activity was already reported for the extracts of ME exhibited significant activity with different methods. The results of the above studies showed significant antioxidant activity for alcoholic extracts of ME and also with ethyl acetate and methanol fractions of ME. In the DPPH method, the alcoholic extracts, ethyl acetate and methanol fraction of ME showed potent antioxidant activity, with IC_{50} values ranging from 20-60 µg/ml. However, the standards rutin and ascorbic acid exhibited better results with lower IC_{50} values. In the H_20_2 method, the alcoholic extracts and methanol fraction showed potent activity which is comparable with the standard. In the ABTS method, all the extracts and fractions of ME exhibited potent antioxidant activity. In the p-NDA method, the alcoholic extracts and methanol fractions of ME exhibited antioxidant activity. All the extracts and fractions of ME exhibited moderate or potent antioxidant activity.

DISCUSSION

Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. The production of auto antigen in certain arthritic disease may be due to *in vivo* denaturation of protein. The mechanism of denaturation propably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. From the above results the plants extracts/fractions which showed significant activity were selected for *in vivo* biological studies [8].

Free radical and reactive oxygen species are well known inducers of cellular and tissue pathogenesis leading to several human diseases, such as cancer, inflammatory disorders, rheumatoid arthritis, and diabetes mellitus, as well as in the aging process. Many plant species with antioxidant activities act as protective agents against these diseases. The potent antioxidant activity was observed using many methods for all extracts/fractions of both the plants. From the above results, the both the plant extracts/fraction exhibited potent antioxidant activities[15].

From the above *in vitro* studies the alcoholic extracts of ME and ethyl acetate and methanol fraction of ME were selected for phytochemical and biological studies to know the phytoconstituents responsible for the above activity.

CONCLUSION

The above *invitro* studies concluded that extracts and fractions showed moderate or potent antioxidant and anti arthritis activity. Further these extracts and fractions of *Merremia emarginata* were used for *invivo* activity

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