Phytochemical Analysis, Cytotoxic And Antioxidant Potential Of *Ipomoea Pes Caprae*(L)R.Br And *Merremia Umbellata*(L.)H. Hallier.

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Abstract: *Ipomoea pes-caprae* and *Merremia umbellata* initially belonged to the same genus Ipomoea, later both were separated into different genus on the basis of morphological differences. In this study both the medicinal plants were compared using phytochemical analysis, cytotoxic activity and antioxidant potential. Phytochemical tests revealed lots of similarities than variation. The only difference in case of phytochemical analysis of the two plants was in the detection of tannins which was present in all the samples of *Ipomoea pes-caprae* while it was present only in polar extract in *Merremia umbellata.* In spectral analysis most of the extracts showed similar profile, except for hexane extracts. Majority of peaks were similar with a minor peak here or there. Regarding cytotoxic activity *Ipomoea pes caprae* showed better results(more than 80%) as compared to *Merremia umbellata* which showed less than 75% cytotoxic activity. *Ipomoea pes capre* revealed marginally better antioxidant properties in comparision to *Merremia umbellata.*

Keywords: *Ipomoea pes-caprae, Merremia umbellata,* cytotoxic, antioxidant, spectral analysis, DPPH assay, FRAP assay

1. INTRODUCTION

Ipomoea pes-caprae and *Merremia umbellata* belong to family Convolvulaceae. Earlier both the plants belonged to one genus that is Ipomoea but advanced members of this family were placed in separate genus Merremia on the basis of morphological differences. Both the species have been used in folk remedies for one ailment or the other. Studies on crude extract of *Ipomoea pes-caprae* resulted in the isolation of two antispasmodic compounds namely beta-damascenone and E-phytol [1]. Plant has also shown hypoglycemic potential [2]. Antioxidant properties have also been reported from *Ipomoea pes-caprae* against free radical scavenging models [3]. *Merremia umbellata* has been reported for allelopathic potential against germination of Arabidopsis seeds and 8 molecules were isolated based upon this assay [4]. *Merremia umbellata* has been widely used in folk remedies. Poultice of leaf is applied on burns and sours [5].

2. MATERIALS AND METHODS

2.1 Plant material. Leaves of *Ipomoea pes-caprae* and *Merremia umbellata* were collected from medicinal germplasm garden of Regional Plant Resource Centre, Bhubaneswar. Fresh and dried weight of leaves is taken to calculate the moisture content of the samples.

$$\% \text{ Moisture content} = \frac{\text{Wet weight of leaves} - \text{Dried weight of leaves}}{\text{Wet weight of leaves}} \times 100$$

2.2 Solvent extraction Crude methanolic extraction was prepared by cold maceration technique followed by its partition into one polar and one non polar solvents. Crude methanol extract obtained as above was subjected to liquid/ liquid separation using hexane (non- polar) as solvent. Upper phase consisting of hexane and lower phase consisting of methanol was separated using a separating funnel. Both the extracts obtained were concentrated in Bucchi (R-200) Rotavapour. Concentrated extract were stored in screw cap vials until further use.

2.3 Phytochemical analysis Extracts of both the plants were investigated for the presence of major class of compounds like alkaloids, flavonoids, saponins, tannins and glycoside as described by Harborne[6]

2.4 Spectral analysis 1gm of fresh leaves of both the plant *Ipomoea pes-caprae* and *Merremia umbellata* were extracted using four different solvents hexane, acetone, chloroform and methanol respectively. Spectral analysis of all the freshly prepared extracts was conducted using UV-Vis spectrophotometer (Specord 200). The results obtained were represented in tabular form and graphs were plotted using wavelengths in X-axis and absorbance in Y- axis for each extract.

2.5 Cytotoxic activity Cytotoxic activity was assessed by using brine shrimp mortality assay[7], LC100 of all the extracts of both the medicinal plants was compared.

2.6 Antioxidant activity Antioxidant activity was detected using qualitative and quantitative assays. For qualitative assay, TLC based DPPH radical scavenging assay was conducted [8]. For quantitative assay ferric reducing power assay and DPPH radical assays were carried out [9,10]. Ascorbic acid was used as reference antioxidant.

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3. RESULTS AND DISCUSSIONS

3.1 Moisture content Moisture content of both plants *Ipomoea pes-caprae* and *Merremia umbellata* was 82.3 and 72.6% respectively, although both the creepers were growing side by side under the same conditions there was a marked difference in their moisture content. This could be due to the different texture of leaves as the leaves of *Ipomoea pes-caprae* are more fleshy and succulent as compared to the leaves of *Merremia umbellata.*

3.2 Phytochemical analysis Both the medicinal plants were similar in the fact that both were devoid of Alkaloids, anthraquinone, flavonoids, glycosides and phenolics (Table 1). In an earlier study phenolics have been reported from *Merremia umbellata* [4], study supported the presence of phenolics in fresh samples of the plant. However, in other two extracts same was not found. Saponin was present in all the samples, where as terpenoids was present only the fresh sample of both the plants suggesting that chemical extraction renders terpenoid as null. The only difference in case of phytochemical analysis of the two plants was in the detection of tannins which was present in all the samples of *Ipomoea pes- caprae* while it was present only in polar extract in *Merremia umbellata.* Comparative analysis of both the plants showed more similarity than disparity with one other. Regarding the presence of terpenoids in fresh sample is that it is labile in nature and hence any chemical extraction removes it from the extracts.

Table 1. Phytochemical analysis of selected medicinal plants.

Class of compounds	*Ipomoea pescapre*		*Merremia umbellata*	
	Non polar extract	Polar extract	Non polar extract	Polar extract
Alkaloid	-ve	-ve	-ve	-ve
Tannin	+ve	+ve	-ve	+ve
Saponin	+ve	+ve	+ve	+ve
Anthraquinone	-ve	-ve	-ve	-ve
Glycosides	-ve	-ve	-ve	-ve
Phenolics	-ve	-ve	-ve	-ve
Terpenoids	-ve	-ve	-ve	-ve
Flavonoids	-ve	-ve	-ve	-ve

3.3 Spectral analysis
Spectral analysis of the two medicinal plants was conducted for both the abstracts. As can be seen by the spectra of non-polar extract(A1,B1) of the two it is evident that upto the range of 0-500nm spectra is almost similar after 500nm to 900 nm there is marked absence of two peaks present at 650nm and 700nm(Fig.1).

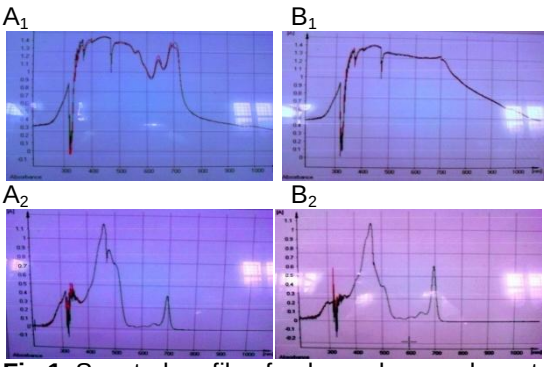
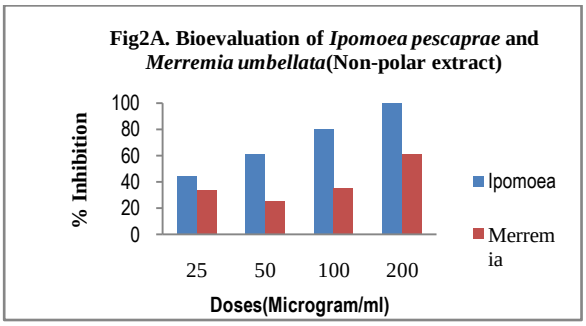
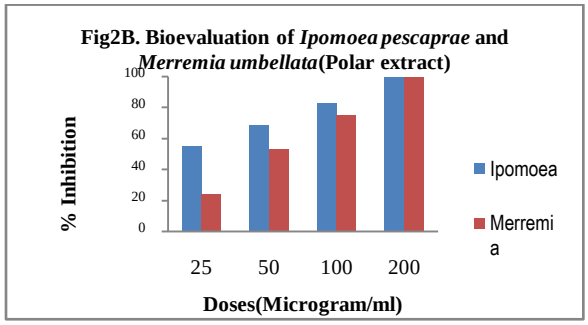


Fig 1. Spectral profile of polar and non polar extracts of Ipomoea pescapre(A) and Merremia umbellata(B) leaf
1= Non polar 2=Polar

3.4 Bioevaluation of solvent extracts
For biological evaluation two parameters were selected these were cytotoxic activity using brine shrimp assay and antioxidant activity by qualitative (TLC based) and quantitative analysis (DPPH radical scavenging and FRAP assays).

3.41Cytotoxic activity using brine shrimp assay: All the extracts were tested in four doses (25, 50 and 100 µg/ml). Details are shown in Fig 2. *Ipomoea pes caprae* showed more activity at all the doses in non polar extracts (A). *Ipomoea pes-caprae* was more active at lower doses but at the dose of 200microgrm/ml dose both had similar activity(100%) in case of polar extract(B). This was supported by the earlier studies in which a number of Ipomoea species have shown good cytotoxic and anti-inflammatory activity [11, 12]. Brine shrimp assay is a very good model for detecting anticancer and anti- inflammatory activity as a good correlation of the model with antitumor and anticancer activity has been reported by a number of workers [13]. At higher doses it can be seen that both have shown good results in non polar and polar extracts, which indicates that though genus of the two plants varies but as far as their medicinal usage is concerned they have significant potential. A significant activity of crude and polar extract of these plants establishes it to be a promising medicinal plant and needs an elaborate study for exploring its anti- inflammatory or anticancer potential.





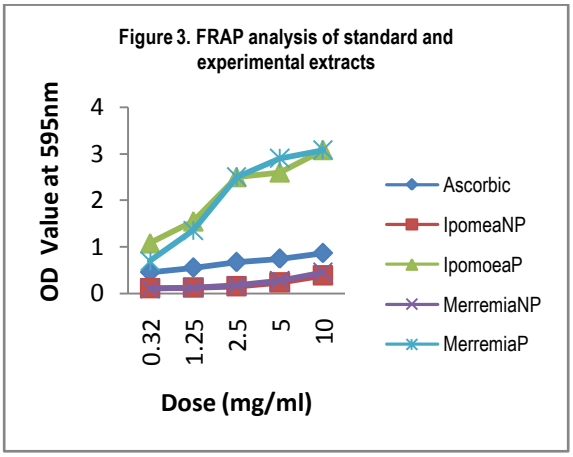
3.42 Antioxidant activity of Ipomoea pes-caprae and Merremia umbellata

Qualitative assay(TLC based) Maximum number of antioxidant bands was found in *Merremia umbellata's* non polar extract suggesting it to be the best as far the antioxidant potential is concerned (Table 2). RF values of the bands were not similar suggesting that the molecules responsible for the conversion of radical DPPH to hydrazine were different in both the plants.

Table 2: TLC based antioxidant assay of *Ipomoea pes-caprae* and *Merremia umbellata* extracts.

Solvent extract used for TLC	***Ipomoea pescaprae***		***Merremia umbellata***	
	Number of antioxidant bands			
	Non polar extract	Polar extract	Non polar extract	Polar extract
Ethyl acetate: methanol: water (40:5.4:4)	1	4	2	4
Chloroform: ethyl acetate: formic acid (5:4:1)	3	7	3	6
Benzene: ethanol: ammonium hydroxide (90:10:1)	7	4	12	1

Quantitative antioxidant assay In case of DPPH radical scavenging assay only polar extract of *Merremia umbellata* showed some activity but as compared to the standard values same was not significant. This result failed to support the qualitative assay in which non polar extract was active. Although Ipomoea species have been reported to have antioxidant potential[14] , results of DPPH assay were contrary to the reports. Second quantitative assay i.e., FRAP assay proved to be more in tune with the earlier reports, here polar extracts of both the species were better than the standard ascorbic acid(Figure 3). Thus, study has provided promising leads for isolation of cytotoxic as well as antioxidant molecules from both the species.



4. CONCLUSION

Merremia umbellata and *Ipomoea pes caprae* belong to a common family Convolvulaceae. Earlier *Merremia umbellata* was known as *Ipomoea umbellata*, so it belonged to the genus Ipomoea, but now the advanced members of Ipomoea were separated and another genus Merremia was recognised. In this dissertation thesis an effort was made to study the similarity and differences in the two species on the basis of phytochemical analysis and bioevaluation. Moisture content of the two species varied by a margin of 10 percent indicating a morphological alteration in the leaves of the two species. Phytochemical tests revealed lots of similarities than variation. The only difference in case of phytochemical analysis of the two plants was in the detection of tannins which was present in all the samples of *Ipomoea pes- caprae* while it was present only in polar extract in *Merremia umbellata.* In spectral analysis most of the extracts showed similar profile, except for hexane extracts. Majority of peaks were similar with a minor peak here or there. Regarding cytotoxic activity *Ipomoea pes caprae* showed better results(more than 80%) as compared to *Merremia umbellata* which showed less than 75% cytotoxic activity. Overall both the species, though now belong to different genus, yet they are potential candidates in the field of drug development.

ACKNOWLEDGEMENT

The authors wishes to thank Forest and Environment Dept. Govt. of Orissa for providing support for the studies.

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