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Evaluation of phytochemical contents of *Ipomoea cairica* (L) Sweet – a qualitative approach

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ABSTRACT

Phytochemical screening was performed on leaves and flower extracts of *Ipomoea cairica* obtained from Aizawl, Mizoram. To analyse the bioactive compounds, Pet. ether, $CHCl_3$ and CH_3OH extracts of the selected plants were tested by following standard procedures. The results demonstrated the presence of alkaloids, sterols, flavonoids, reducing sugars, tannins, saponins, terpenoids, anthraquinones, glycosides and phenols. These results were compared with literature values. Indications from the results depicted usefulness of the plant parts in the treatment of some common diseases.

Key words: *Ipomoea cairica*, Bioactive compounds, CHCl3, CH3OH, petroleum ether.

INTRODUCTION

Common name : Mile a minute vine, Messina creeper, Cairo morning glory, Coast morning glory and Railroad creeper.
Family : Convolvulaceae
Species : *Ipomoea cairica*
Synonyms : *Ipomoea palmata* Forssk, *Ipomoea stipulacea* (Jacq.)

Convolvulaceae comprise nearly 1650 predominantly tropical species. The genus *Ipomoea* with approximately 500 – 600 species comprises the largest no. of species within the Convolvulaceae.[1,2] This family is dominated by twining and climbing woody or herbaceous plants that often have heart- shaped leaves and funnel-shaped flowers.[3] The genus *Ipomoea* occurs in the tropics of the world although some species also reach temperate zones.[5] The species of this genus are mainly distributed throughout the South and Central America countries and Tropical African territories.[2] One of the most noticeable characteristics of the Convolvulaceae is the existence of cells which secrete resin glycosides in the foliar tissues and in the roots of the plants. These glycoresins constitute one important chemotaxonomic marker of this family[28] and are responsible for the purgative properties of some species of this family.[20] Convolvulaceae are found throughout tropical and subtropical regions of the world. Several species of the genus

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Ipomoea, as well as, of the Convolvulaceae family have the property of phytotoxicity, which mean suppressing the growth of other plants including invasive weeds[15]. *Ipomoea cairica* L. Sweet is used in Brazilian folk medicine for the treatment of rheumatism and inflammations through inhibition of the release of mediators induced edema[12].

In Mizoram, there is no report on the traditional use of *Ipomoea cairica* as a medicine or as a pesticide and is considered only as a weed.

Materials and Methods

The plants were collected from the areas in and around Mizoram University, Mizoram and were identified by Botanical Survey of India, Shillong, Meghalaya (No.BSI/ERC/2012/Plant identification/ dated 28-8-2013), coll no. 2 *Ipomoea cairica*. Voucher specimens were kept in the Department of Botany, Pachhunga University College, Aizawl, Mizoram for future reference.

The plant was selected with an aim to provide the local people a safe, cheap and easily available plant based insecticides and/or pesticides for use in their households and agricultural lands because keen observation of these plants over ages reveals that these plants though abundantly found had not been attacked by most insect pests. It is therefore believed these properties might be attributed to the presence of some known /unknown bioactive compound(s) present in them which possess pesticidal and/or insecticidal properties.

The collected leaves and flowers of *Ipomoea cairica* were cleaned, washed with distilled water and dried under shade with occasional shifting and then powdered with an electrical grinder. These powders were stored in air sealed brown bottles at ambient temperature. The dried, powdered materials of (3.5 kg) and flowers (2.8 kg) were sequentially extracted starting with pet. ether/n-hexane to remove lipids, oils and fats followed by exhaustive extraction with $CHCl_3$ for removal of chlorophyll and its derivatives. The plant materials were then finally extracted continuously by soxhlet extractor and maceration with CH_3OH. Phenolics with only few hydroxyl groups are soluble in ether, $CHCl_3$, EtOAc, CH_3OH, and EtOH[27]. Methanol, ethanol, water, and alcohol-water mixtures are most commonly used for dissolving phenolic compounds for analytical purposes. The extractions were repeated for many times and the combined extracts were filtered and concentrated in a vacuo using a rotary evaporator at reduced pressure (22–26 mm Hg) and low temperature of 45° C to collect the crude extract and to remove the last traces of the solvents. A dark green coloured (for leaves) and dark-red coloured semi- solid mass (for flowers) were obtained after concentration. The concentrated extracts were kept in refrigerator at 4°C for further use. The percentage yields of CH_3OH extract of *Ipomoea cairica* flowers was found to be 3.97 % w/w. The pet. ether, $CHCl_3$ and CH_3OH extracts were subjected to preliminary phytochemical analysis to identify the presence of phytoconstituents.

Pet. ether, $CHCl_3$ and CH_3OH extracts of the selected plants were tested by following standard procedures described below, for the presence of phytochemical constituents such as alkaloids, steroids, flavonoids, gums, reducing sugars, tannins, saponins, terpenoids, aminoacids, anthraquinones, cardiac glycosides and phenols. Table 1 gives the results of preliminary phytochemical group testing of the crude extracts. Reagents used for the different chemical group tests were prepared as per standard protocols as follows:

- Mayer's reagent: Solution of 1.36gm of mercuric iodide in 60ml of water was mixed with a solution containing 5gm of potassium iodide in 20ml of water.
- Libermann-Burchard reagent**:** 5gm of acetic anhydride was mixed under cooling with 5ml of conc. H_2SO_4 and was added slowly to 50ml of absolute ethanol with cooling.
- Dragendroff reagent: 1.7gm basic bismuth nitrate and 20gm tartaric acid were dissolved in 80ml of water. This solution was mixed with a solution containing 16gm potassium iodide in 40ml of water.
- Fehling's solution A: 34.64gm copper sul-

phate was dissolved in a mixture of 0.5ml of sulfuric acid and sufficient water to produce 500ml.

- Fehling's solution B: 176gm of sodium potassium tartarate and 77gm of sodium hydroxide were dissolved in sufficient water to produce 500ml. Equal volumes of above solutions (Fehling's solution A and B) is mixed at the time of use.
- Benedict's reagent: 1.73gm of cupric sulphate, 1.73gm of sodium citrate and 10gm anhydrous sodium carbonate were dissolved in water and the volume is made up to 100ml with water.
- Molish's reagent: 2.5gm of pure α-naphthol was dissolved in 25ml of ethanol.

Test for Alkaloids[22]

- Mayer's Test: 1 to 2 ml of extract was taken in a test tube. 0.2 ml of dil. HCl and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff coloured precipitate gives positive test for alkaloid.
- Dragendroff's Test: 0.1ml of dil. HCl and 0.1 ml of Dragendroff's reagent were added in 2 ml solution of extract in a test tube. Development of orange brown coloured precipitate suggested the presence of alkaloid.
- Wagner's Test: 2 ml of extract solution was treated with dil. HCl and 0.1 ml of Wagner's reagent. Formation of reddish brown precipitate indicated the positive response for alkaloid.
- Hager's Test: 2 ml of extract was allowed to react with 0.2ml of dil. HCl and 0.1 ml of Hager's reagent. A yellowish precipitate suggested the presence of alkaloid.

Test for Amino acids[26]

- Ninhydrin Test: Extract solution was treated with Ninhydrin (Tri-ketohydrindene hydrate) at the pH range of 4 - 8. Development of purple color indicated the positive response for amino acids.

Test for Anthraquinones[9,13,24]

- Modified Borntrager's Test: 5 ml of extract solution was hydrolyzed with dilute sulphuric acid and extracted with benzene. 1 ml of dilute ammonia was added to it. Rose pink coloration suggested the positive response for anthraquinones.

Test for Reducing sugars[13, 26]

- Fehling's test for free reducing sugar: About 0.5 g of extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B.

Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

- Benedict's Test: To 5 ml of the extract solution, 5 ml of Benedict's solution was added in a test tube and boiled for few minutes. Development of brick red precipitate confirmed the presence of reducing sugars.

Test for Flavonoids[4,26]

- Shinoda test: About 0.5 g of each extract portion was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.
- Sulphuric acid test: A fraction of the extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour.
- NaOH test: A small amount extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

Test for Gums

- Molisch's Test: 2 ml of concentrated sulphuric acid was added to 2 ml of extract solution. Then it was treated with 15% α-naphthol in ethanol (Molisch's reagent). Formation of a red violet ring at the junction of two layers indicated the positive test for gums.

Test for Saponins[13, 26]

- Foam Test: A small amount of extract was shaken with water and looked for the formation of persistent foam.

Test for Sterols[13, 26]

- Liebermann-Burchard test: One ml extracts was treated with chloroform, acetic anhydride and added drops of H_2SO_4 and observed for the formation of dark pink or red colour.
- Sulphuric acid test: The fraction of extract was treated with ethanol and H_2SO_4 and observed for the formation of violet blue or green colour.

Test for Tannins[9,13]

- $FeCl_3$ Test: 5 ml of extract solution was allowed to react with 1 ml of 5% ferric chloride solution. Greenish black colouration indicated the presence of tannins.
- Pot. Dichromate Test: 5 ml of the extract was treated with 1 ml of 10% aqueous potassium dichromate solution. Formation of yellowish brown precipitate suggested the presence of tannins.
- Lead acetate Test: 5 ml of the extract was treated with 1 ml of 10% lead acetate solution in water. Yellow colour precipitation gave the test for tannins.

Test for Phenols[9,13]

- Ferric chloride test: A fraction of extract was treated with 5% ferric chloride ,formation of deep blue colour confirms the presence of phenol
- Liebermann's test:The extracts was heated with sodium nitrite, add H_2SO_4 solution diluted with water and add excess of dilute NaOH and observed for the formation of deep red or green or blue colour.

Test for terpenoids[13, 26]

- Chloroform test: The plant extract was taken in a test tube with few ml of chloroform and add concentrated sulphuric acid carefully to form a layer and observed for presence of reddish brown colour.
- Liebermann-Burchard test: 1 ml extracts was treated with chloroform, acetic anhydride and added drops of H_2SO_4 and observed for the formation of dark green colour.

Test for Glycosides[22]

- Legal's test: Dissolved the extract (0.1 g) in pyridine, added sodium nitroprusside reagent and made alkaline with NaOH solution. Pink to red colour solution indicates the presence of glycosides.
- Borntrager's test: The extract is hydrolyzed with concentrated HCl for 2 hours on a water bath and filtered and few ml of above filtrate was shaken with chloroform, chloroform layer was separated and added 10 % ammonia, formation of pink colour indicates the presence of glycosides.

RESULTS

The results of phytochemical group tests for all the crude extracts of the plant under study were provided in Table 1. The results demonstrated the presence of alkaloids, sterols, flavonoids, reducing sugars, tannins, saponins, terpenoids, anthraquinones, glycosides and phenols. In the leaves of *Ipomoea cairica,* terpenols and glycosides are present in its petroleum and $CHCl_3$ fractions, sterols in petroleum and CH_3OH fractions while reducing sugars and anthraquinones are exclusively present in the $CHCl_3$ fraction. The CH_3OH extract also contains alkaloids, flavonoids, tannins, saponins, anthraquinones and phenols. The flower extract of *Ipomoea cairica* contains sterols (all extracts), flavonoids, tannins, saponins and phenols (CH_3OH extract), reducing sugars ($CHCl_3$ and CH_3OH), and terpenoids and glycosides (both in petroleum and $CHCl_3$ fractions).

Table 1. Results of phytochemical screening of plant extracts.

Sl. No	Plant Constituents	Test/ Reagent	*Ipomoea cairica* leaves			*Ipomoea cairica* flower		
			Pet ether.	$CHCl_3$	CH_3OH	Pet ether.	$CHCl_3$	CH_3OH
1.	Alkaloids	Mayer	-	-	+	-	-	-
		Dragendorff	-	-	+	-	-	-
		Wagner	-	-	+	-	-	-
		Hager	-	-	+	+	-	-
2.	Sterols	Liebermann-Burchard	+	-	+	+	+	+
		H_2SO_4	+	-	+	+	+	+
3.	Flavonoids	Shinoda	+	+	+	-	-	+
		H_2SO_4	-	-	+	-	-	+
		NaOH	-	-	+	-	-	+
4.	Gums	Molisch	-	-	-	-	-	-
5.	Reducing sugars	Fehling	-	+	-	-	+	+
		Benedict	-	+	-	-	+	+
6.	Tannins	Pot. Dichromate	-	-	+	-	-	+
		Lead acetate	-	-	+	-	-	+
		$FeCl_3$	-	-	+	-	-	+
7.	Saponins	Foam	-	-	+	-	-	+
8.	Terpenoids	$CHCl_3$ Liebermann-Burchard	+ +	+ +	- -	+ +	+ +	- -
9.	Anthraquinones	Borntrager Upper layer	-	+	+	-	-	-
		$CHCl_3$ layer	-	+	+	-	-	-
10.	Glycosides	Legal	+	+	-	+	+	-
		Borntrager	+	+	-	+	+	-
11.	Phenols	$FeCl_3$	-	-	+	-	-	+
		Liebermann	-	-	+	-	-	+
12.	Amino acids	Ninhydrin	-	-	-	-	-	-

+ Present; - Absent

DISCUSSION

The phytochemical compounds observed in the extracts of the plants are known to play important roles in bioactivity of medicinal plants and these secondary metabolites exert antimicrobial activity through different mechanisms. The medicinal values of medicinal plants lie in these phytochemical compounds, and as such, produce definite physiological actions on the human body.

Tannins, which are part of the phytochemical constituents, have been found to form irreversible complexes with prolinerich protein[23] resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to pro-

vide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery[6]. These observations therefore support the use of *I. cairica* in herbal cure remedies. Li and Wang (2003) reviewed the biological activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *I. cairica* has potential as a source of important bioactive molecules for the treatment and prevention of cancer.

Another secondary metabolite compound observed was alkaloid. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines[17]. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications[11].

It is documented that the presence of Saponins can control human cardiovascular disease and reduce cholesterol, also tannins may provide protection against microbiological degradation of dietary proteins in the semen[1]. Just et al. (1998) revealed the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in CH_3OH extracts of both leaves and flower extracts and has supported the usefulness of this plant in managing inflammation.

Steroidal compounds present in the extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones[18]. Quinlan et al. (2000) worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Neumann et al. (2004) also confirmed the antiviral property of steroids.

Flavonoids, another constituent of *I. cairica* leaves and flower extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti -allergic, cytostatic and antioxidant properties[8]. One of the ability of flavonoids is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals and thus health promoting in action[7].

The presence of glycosides also reveals the anti-diarrhoeal nature of the extracts[25].

Conclusion

The results of the phytochemical screening of *Ipomoea cairica* crude extracts from leaves and flowers showed that the samples contained some bioactive substances. The leaves and flowers can be used in prevention or curing some major diseases since the results show therapeutic compositions.

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References

1. Aletor VA (1993). Allelochemicals in plants and feedingstuffs: 1. Nutritional, biochemical and physiopathological aspects in animal production. *Vet Hum Toxicol*, **35**, 57 – 67.
2. Austin DF & Huaman Z (1996). A synopsis of *Ipomoea* (Convulvulaceae) in the Americas, *Taxon*, **45**, 3-38.
3. Austin DF (1997). Convolvulaceae (morning glory family). *Ag.arizona.edu/herbarium/assoc/people/daustin/convolve.html*, (accessed 2011).
4. Ayoola GA, Coker HAB, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC & Atangbayila TO (2008). Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in southwestern Nigeria. *Trop J Pharm Res*, **7 (3)**, 1019-1024.
5. Cao S, Guzza RC, Wisse JH, Miller JS, Evans R & Kingston DGI (2005). Ipomoeassins A-E, cytotoxic macrocyclic glicoresins from the leaves of *Ipomoea squamosa* from

the Suriname rainforest, *J Nat Prod*, **68**, 487-492.

6. Dharmananda S (2003). Gallnuts and the uses of Tannins in Chinese Medicine. In: Proceedings of Institute for Traditional Medicine, Portland, Oregon.
7. Ferguson LR (2001). Role of plant polyphenols in genomic stability, Mutat Res, 475, 89-111.
8. Hodek P, Trefil P & Stiborova M, (2002). Flavonoids - Potent and versatile biologically active compounds interacting with cytochrome P450, *Chemico-Biol Intern*, **139(1)**, 1 -21.
9. Jeffrey B (2007). Phytochemical methods, a guide to modern techniques of plant analysis.
10. Just MJ, Recio MC, Giner RM, Cueller MJ, Manez S, Bilia AR & Rios JL (1998). Anti-inflammatory activity of unusual lupine saponins from *Bupleurum fruticescens*, *Planta Medica*, **64**, 404-407.
11. Kam PCA & Liew (2002). Traditional Chinese herbal medicine and anaesthesia, Anaesth, 57(11), 1083-1089.
12. Karawya MS, Amar NM, Hifnawy MS, Al-Okbi SY, Mohamed DA & El-Anssary AA (2010). Phytochemical study and evaluation of the antiinflammatory activity of some medicinal plants growing in Egypt, *Med J Islam World Acad Sci*, **18(4)**, 139-150.
13. Kokate CK (1994). Practical pharmacognosy, Ed 4, Vallabh prakashan.
14. Li D & Wang P (2003). Antifungal activity of Paraguayan plant used in traditional medicine, *J. Ethnopharmacol*, **76**, 93 -98.
15. Meira M, da Silva EP, David JM & David JP (2012). Review of the genus *Ipomoea*: traditional uses, chemistry and biological activities, *Revista Brasileira de Farmacognosia*, **22 (3)**, 682-713.
16. Neumann UP, Berg T, Baha M, Puhl G, Guckelbeger O, Langreh JM & Neuhaus P (2004). Long-term outcome of liver transplant for hepatitis C: A 10 year follow-up. *Transplan*, **77 (2)**, 226-231.
17. Nobori T, Miurak K, Wu DJ, Takabayashik LA & Carson DA (1994). Deletion of the cyclin-dependent kinase-4 inhibitor gene in multiple human cancers, *Nature* **368 (6473)**, 753-756.
18. Okwu DE (2001). Evaluation of the chemical composition of indigenous Spices and flavouring agents, *Global J Appl Sci*, **7(3)**, 455-459.
19. Parekh J & Chanda S (2007). In vitro antibacterial activity of crude methanol extract of *Woodfordia fruticosa* Kurz flower (Lythacease), *Braz J Microbiol*, **38**, 2.
20. Pereda-Miranda R & Bah M (2003). Biodynamic constituents in the mexican morning glories: purgative remedies transcending boundaries, *Curr Top Med Chem*, **3**, 111-131.
21. Quinlan MB, Quinlan RJ & Nolan JM (2000). Ethnophysiology and herbal treatments of intestinal worms in Dominica, West Indies. *J. Ethanopharmacol*, **80**, 75-83.
22. Raaman N (2008). Phytochemical Techniques, New India Publishing Agency.
23. Shimada T (2006). Salivary proteins as a defense against dietary tannins. *J Chem Ecol*, **32(6)**, 1149-1163.
24. Sofwara A (1982). Medicinal plants and medicine in Africa, John Wiley & Sons Ltd., Chichester, pp.142.
25. Tiwari P, Kumar B, Kaur M, Kaur G & Kaur H. (2011). Phytochemical screening and Extraction: A Review, *Intern Pharm Sci*, **1**,1.
26. Trease GE & Evans WC (1983). Text book of pharmacognosy, 12th Edition, Balliere Tinad, London, pp. 257.
27. Van Sumere CF (1989). Phenols and phenolic acids. In: *Methods in Plant Biochemistry, Vol (1) Plant phenolics* (PM Dey & JB Harbourne, eds), Academic Press, London, pp. 29-73.
28. Wagner H (1973). The chemistry of the resin glycosides of the Convolvulaceae family, In: *Medicine and natural sciences, chemistry in botanical classification* (G Bendz & J. Santesson, eds.), Academic Press, New York, pp. 235-240.