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Phytochemical investigation of *Hygrophila auriculata* and *Leucas aspera:* Weeds

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Abstract
Asteracantha longifolia and *Leucas aspera* are ragrded as weeds they grows in swampy, marshy waterlogged and dry, open, sandy soil where there is no human intervention. In this present study, we have performed phytochemical analysis to validate their phytochemical constitution. The obtained results supports us to say that the plants were having therapeutic principles. The purpose of writing this article is to pour limelight on the application part of weeds which are available in the rural premises to cater the unmet health needs of farming communities, for the sustainable maintenance of livelihood in the rural setup as well as the conservation of biological resources.

Keywords: Weed, biodiversity, phytochemicals, *Hygrophila auriculata, Leucas aspera*

1. Introduction
Therapeutical/biologically active ingredients in the medicinal plants have made them a primary source of medicine since ancient times/ time immemorial. Even, today these medicinal plants were used by tribes, traditional healers in the form of folk medicine they found it effective against specific disorders all across the globe. In contrast, most of the modern synthetic medicine structures were derived from these medicinal plants. Most of the medicinal plants/herbs mainly grow in forest area, because they tend to prefer undisturbed ecosystem, which leads to rich biodiversity and endemism. Because of an unscientific collection of these Medicinal and Aromatic Plants (MAPs) by the collectors leading to thinning of forest resources [10]. Some legal frameworks from the government agencies have been implemented to limit/prohibit the collection of MAPs, few centralised bodies like Botanical Survey of India and CIMAP have been authorised to have a repository of some endangered MAPs. In view of this, sustained supply and conservation of the MAPs becomes utmost important, on the other hand, it is advisable to look for the other plants which are regarded as a nuisance in agro-ecosystems i.e., WEEDS and explore their benificial aspects interms of fodder, medicinal and household.
The present study deals with two such weeds *Hygrophila auriculata* (L.) Nees and *Leucas aspera* which were collected near Shivamogga city of Karnataka State, India, the former one affluently grows in water logged places like beside of lakes, trenches around the paddy fields and the latter one grows well in open, dry and sandy soil with minimum moisture.
Hygrophila auriculata also known with few synonyms *viz, Asteracantha longifolia Hygrophila spinosa, Hygrophila shulli*. In Sanskrit, it is called as *Ikshura, Ikshuragandha, Kokilaksha* and in Kannada, it is called as *Nirmuli, Kolavanki gida*. It is an annual stout plant growing up to 1.5 meters in height, bearing 2-3cm long yellowish brown spines/thorns at nodes and internodes. Leaves are ranging from oblong to lanceolate. Blooms in the month of November to December with violet coloured flowers, after fertilisation which bears two-celled fruit covered with tiny hair-like structures, accommodate 4-8 seeds. Seeds are ovate (flat/compressed) and 0.2-0.25cm in length [2]. Kokilaksha uses were referred in several rasayanas and also in Vagbhatacharya's *Ashtang hr da yam* text for the cure of rheumatism and arthritis. Few researchers have found that the different solvent extract of the plant is having antioxidant, antibacterial against few clinical isolates, haematopoietic, anti-cancer, hepatic and cardioprotective effects [8]. In our study, we have studied this whole plant with a holistic approach to restoring its medicinal uses and a simple domestic method to extract a therapeutically active crude extract to use it as emergency medicine.
Leucas aspera commonly known as 'Thumbe gida' in Kannada and in Sanskrit it is called as Dronapushpi.It is a common herb which is belongs to Lamiaceae family [5]. As per the Botanical Survey of India it is found through out the country irrespective agroclimatic conditions and few crop researchers have regarded this herb as a weed as it grows along the sides of crop lands and sometime in between the crops. The indigenous folklore listed following medicinal use of this plant; antimicrobial, against cold and cough in children, hypoglycemic with some other medicinal herbs, used in scorpion bites as anti-dote, natural insecticide and proven mosquito repellant activity and to treat skin diseases like psoroasis the list will go on [1, 9].

2. Materials and methods
2.1 Collection and preliminary processing of Plant material
A. longifolia and *L. aspera* whole plants were collected at its pre-flowering stage from Lakkinakoppa village, close to Shivamogga city which is lying at the North latitude 13º 27' and 14 º39' and East longitude 74 º 37' and 75 º 52'. Plant authentication was done by Botanical Survey of India, Western Circle, Pune. Collected plants were washed under running tap water to remove dirt, mud and soil particles from roots as well as aerial parts, then the washed plant

material was shade dried for 3-7 days and pulverised to obtain a moderately fine powder for the extraction purpose.

2.2 Extraction of plant material
100 gms of dried powdered plant material of *A. longifolia* and *L. aspera* was boiled in distilled water in a stainless steel vessel for about 1 hour and allowed to cool under room temperature. Then the same extract was concentrated in a rotary evaporator to reduce the volume to 100mL to get a concentration of 1g/ mL, same was stored in glass jar for further use.

2.3 Phytochemical screening [3, 4, 6, 7, 12]
2.3.1 Test for Carbohydrate
Benedicts tests: 1 ml of extract is mixed with Benedicts reagent and boiled for 5mins. Formation of brick red precipitate indicates the presence of carbohydrate.

2.3.2 Test for alkaloids
15 mg of the extract was stirred with 1% HCl (2-5 ml) in a water bath for 5 min and filtered. These filtrates were divided into two equal parts.

a. *Dragendorff's test***:** add 5 ml of distill water and 2 ml of HCl for 2ml of extract and kept for some time, then 1 ml of Dragendorff's reagent is added
b. *Mayer's test:*2 ml of extract was mixed with Mayers reagent (Potassium mercuric iodide solution)

2.3.3 Test for Flavonoids
a. *Shinodas test:* 1 ml of extract is treated with 10 drops of diluted HCl followed by a piece of Magnesium.
b. *Ferric chloride test:* 1 ml of extract is treated with Ferric chloride.

2.3.4 Tests for steroids and terpenoids
a. *Salkowski test:* The crude extract of about 100 mg was separately treated with 2ml of chloroform with intermittent shaking followed by the addition of concentrated H_2SO_4 (2 ml) slowily along the side of the test tube, a reddish brown ring/coloration of the interface indicates the presence of terpenoid.
b. *Liebermann-Burchard test:* Extract (100 mg) was shaken with chloroform in a test tube; few drops of acetic anhydride was added to the test tube and boiled in a water bath and rapidly cooled in iced water. Concentrated H_2SO_4 (2 ml) was slowly added alongside of the test tube. Formation of a brown ring at the junction of two layers and turning the upper layer to green shows the presence of steroids while formation of deep red color indicates the presence of triterpenoids.

2.3.5 Test for tannins
Extract was stirred continueously with distilled water (10 ml) and then filtered. To filtered solution few drops of 5% ferric chloride were then added. Formation of Black or blue-green coloration or precipitate was taken as positive result for the presence of tannins.

2.3.6 Test for Saponins
5 ml of plant extract was treated with sodium bicarbonate with vigorous shaking for 3 to 4 mins, formation of honey comb like froth shows the presence of saponins.

2.3.7 Test for Glycosides
Cardiac glycoside (Keller-Killiani test): Extract (0.5 g) was mixed with distilled water (5 mL). To this mixture, glacial acetic acid (2 ml) containing a few drops of ferric chloride was added, followed by H_2SO_4 (1-2 ml) slowly added along the side of the test tube. Formation of the brown ring at the junction gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

3. Result and Discussion
Aqueous whole plant extracts of both plants have revealed the presence of the series of phytochemicals which are listed in Table.1. *H. auriculata* and *L.aspera* have shown the presence small or minute levels of saponins, terpenoids and steroids and moderate levels of phenolic and carbohydrates. Both were shown the prominent levels of Tannins and flavonoids, but cardiac glycosides was absent in both plants. The results which we have obtained are similar to the the results which is reported by Eazhisaivallabi *et al* [2] and Mandal *et al* [8]. In caase of A. longifolia. Where as *L. aspera* result also resembles the findings of Rahman *et al* [9] and Kamat *et al* [5].

Table 1: Phytochemical screening of H. auriculata and *L. aspera* (+: minute, ++: moderate, +++: good and -: absent)

Type of Phytochemical	Test	*H. auriculata*	*L. aspera*
Carbohydrate	*Benedicts test*	++	+
Alkaloids	*Dragendorff's test*	-	-
	Mayer's test	-	-
Flavonoids	*Shinodas test*	+++	+++
	Ferric chloride test	+	+
Steroids and Terpenoids	*Salkowski test*	++	+
	Liebermann-Burchard test	+	++
Tannins	*Ferric chloride test*	+++	+++
Saponins	*Foam Test*	+	+
Glycosides	*Cardiac glycoside (Keller-Killiani test)*	-	-
Phenols	*Ellagic Test*	++	++

Generally, weeds are growing in the undisturbed ecosystem which is away from any of anthropological activity and they are considered as unwanted plants which interfere in between the agriculture crops. As per herbalists, no plant is useless, according to them there is a need to study some plants where there is a dearth of information regarding its usefulness or harmful effects for documentation purpose [10]. Each and every plant is unique in its chemical entity, say for example if a plant is poisonous in nature it cannot be used for food and fodder, but the same plant can be used for fish poisoning. Hence, it has to be studied in the proper way to determine its poisonous effect as well as making use of the same property for fish poisoning purpose.

For example, Chinese Traditional Medicine (CTM), Sub-Saharan countries, South-America, European Pharmacopeia has separate guidelines for herbal based medicines. This shows that developed as well as developing countries still depends on natural remedies for the well-being of humans and veterinary animals. Presently trading of MAPs fetching a lot of revenue and every year the quantum of the herbal material being traded is gradually increased 5-20% per annum. The number of registered herbal industries in India is close to 10,000 and there is also multiple numbers of unregistered cottage level herbal processing units are into the constant supply of these herbs as raw materials to the industries, this makes use of around 6000 higher plant species for the preparation of herbal medicaments [10]. People who were trading these medicinal plants earlier they didn't know the way of sustainable supply of these herbs. Due to which some of the medicinal plant species are on the verge of extinction. Thorough documentation and validation of the phytochemical profiles in the medicinal plants will definitely help in conserving these beneficial herbs [11].

4. Conclusion

Both H. auriculata and L.aspera they were regarded as weeds, even though they found to have immense medicinal importance. Our study also pour some on their phytochemical profiling and it is evident that they have useful chemical moieties. In depth researh and domestication of the products from these herbs will definitely helps to cater the unmet emergency medicinal needs of rural population and also it will increase the quality of life.

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