

PHYTOCHEMICAL ANALYSIS OF VARIOUS EXTRACTS OF AGERATUM

CONYZOIDES BY USING THE SOXHLET EXTRACTION METHOD

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ABSTRACT

The Asteraceae family includes *Ageratum conyzoides*. The name *Ageratum* comes from the Greek 'ageras,' which means "non-aging," in reference to the plant's exceptionally long lifespan; the species epithet, 'konyz,' is the Greek name for the similar plant, *Inula helenium*. Billy goat weeds, or *Ageratum conyzoides* Linn. (Family Asteraceae, Tribe Eupatorieae), are annual herbs that have been used medicinally for centuries throughout the tropics and subtropics. The entire plant, including the stem and leaves, is covered in tiny white hairs. The average height of this annual herb is about 1 meter. The plant has tiny white hairs all over the stems and leaves. Flowers range from purple to white, and the leaves are oval in shape. *Ageratum conyzoides* has been used in herbal or folk medicine in Africa for a long time as a treatment for a number of different conditions. It has been used as an insect repellent and in alternative medicine for the treatment of epilepsy.

KEY WORDS: Phytochemical Analysis, Extracts, *Ageratum Conyzoides*.

INTRODUCTION

Because of the presence of phytochemical elements, medicinal plants are effective for mending and curing human ailments. Plants' own defense mechanisms and their ability to ward off illness are enhanced by phytochemicals, which are found naturally in medicinal plants, leaves, vegetables, and roots. Primary and secondary phytochemical substances. Secondary chemicals include terpenoid, alkaloids, and phenolic compounds, whereas primary constituents include chlorophyll, proteins, and common sugars. Anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol production, anti-viral, and anti-bacterial actions are just few of the many important pharmacological effects displayed by terpenoids. Terpenoids have a critical role in luring predatory mites that feed on herbivorous insects. Medicinal plants often contain alkaloids that can be extracted and utilized as anesthetics. The fresh leaves are macerated by rubbing them between the palms of both hands; the juice is then pressed into the wound and a bruised but intact leaf is used to cover it. The healing process is supposedly accelerated by applying this dressing once a day. showed that a crude extract of the plant was able to prevent the growth of *Staphylococcus aureus*, a prominent wound pathogen, when cultured in vitro.

There has been a lot of research into the chemical make-up and biological functions of the essential oil found in the plant's leaves and other aerial parts.

There are many reasons in the current situation to switch from synthetic to herbal medicines, which have established themselves as a gift of life. For many years, researchers have been drawn to medicinal plants, which are now highly regarded globally as a rich source of therapeutic substances for the prevention of illnesses and maladies. Humans sought out immediate natural habitat forced eating of plants as a library of therapeutic agents in search of a healthy, lengthened life and treatment to relieve sting. Since there has been a long period of human intrusiveness, there has been an accumulation of knowledge regarding the therapeutic potential of medications. The importance of having medicinal plants and regular health practises in addressing the global fitness heed is growing in popularity. Therefore, there has

been a remarkable increase in new study on plants that contain bioactive chemicals on a global scale. The value of medicinal plants in the traditional health care systems (such as Ayurveda, Unani, Homoeopathy, and Yoga) for treating health issues is receiving more attention. Plant materials and herbal treatments made from them now make up a sizable share of the global health market as a result of this awareness. Herbal medicines rely on traditional knowledge and understanding of plants and their role in herbal medicine to survive. The usage of medicinal plants is one of the oldest, richest, and most distinctive living traditions in India, which is one of the world's top 12 biodiversity hotspots.

Over 20,000 medicinal plants have been identified in our country, 800 of which are used in indigenous Ayurvedic systems. As a result, the Indian subcontinent contains a vast repository of medicinal plants that are employed there. To make medicines and treat various illnesses, many prevalent local systems including Siddha, Ayurveda, Unani, and Allopathy use a variety of natural plants. The primary goals of this method of treatment include strengthening the body and boosting the immune system as well. It is crucial to turn to herbal medicines for the greater good of the suffering humanity and living things of the earth in these times of iatrogenic diseases and excessive prescription prices that cause economic catastrophes in the families of the sufferers.

Remedial plants are those that have components that can be consumed for the restorative purposes of significant medications. Flora is thought of as a molecular factory that produces a huge variety of "bioactive compounds" as byproducts. Since ancient times, medicinal plants have been recognised for their various healing powers. The variety of medicinal plants and their applications for interdisciplinary research, industry growth, and consumer interfaces may contribute to the development of new bio-reactives and the potential for combinational phyto-chemical compounds on innovative pharmacophores. Widespread observation has been made of the use of conventional medicine derived solely from medicinal plants, particularly in underdeveloped nations.

Many ridiculous medical procedures have been used in the past. However, prehistoric knowledge served as the foundation for modern medicine and continues to be a major source of potential treatments and their efficacy. Two broad categories can be used to classify bioactive medications. They are initially included in conventional mixes made up of a variety

of different substances, and then their purity in chemicals with powerful principles is furthered. When a plant's specific medical activity is less or less intense, or when it has a minute medicinal potency, uncontaminated compounds are used.

RESEARCH METHODOLOGY

COLLECTION AND IDENTIFICATION OF EXPERIMENTAL PLANT

Prof. S. Kshetrapal (Dept. of Botany), University of Rajasthan, Jaipur, Rajasthan, India, gathered and identified mature, fresh, healthy, and disease-free plant materials of *Verbesina encelioides* and *Ageratum conyzoides*. For authentication, the University additionally received the voucher specimen numbers *Verbesina encelioides* (*RUBL211585) and *Ageratum conyzoides* (*RUBL211584). Fresh plant material was properly cleaned in tap water, dried in the shade, homogenised into a fine powder, and then stored in airtight bottles for future experiments.

EXTRACTION FROM PLANT MATERIAL

Extraction is the process of separating the mobile extracts from the immobile components by utilising successive solvents in accordance with established methods. The dried powdered plant material is uniformly packed in a round-bottomed flask that is filled with the required solvent, along with a thick filter paper—generally Whatmann filter paper—and placed inside the thimble-shaped equipment. The solvent is then decreased using heat after being put together with a condenser. The solvent starts to evaporate as it moves up to the distillation arm, where it

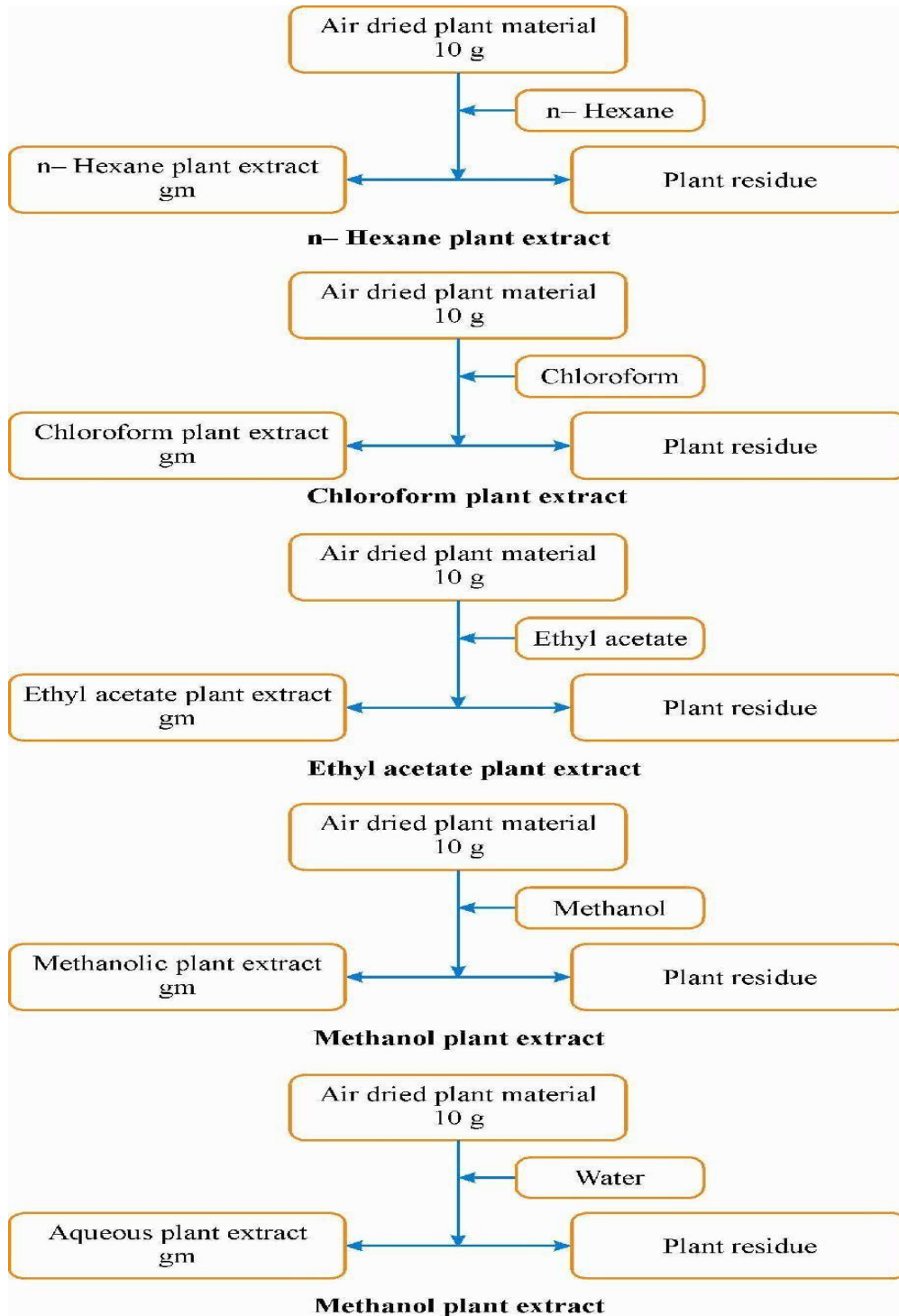


FIG. 1: Flow chart for preparation of plant extracts by Soxhlet extraction method

collects in a chamber with a thimble of powdered material. The evaporating vapours must be made to cool down before collecting in the main collecting chamber, which holds the thimble of solid material.

Finally, the distillation flask is filled with the plant's raw extract. The most crucial aspect of this approach is that it only requires one batch of solvent to isolate bioactive chemicals. A sticky solid residue is produced from the extracted components after the solvent has been removed from the extract using a rotary evaporator. In the current investigation, 200 ml of n-hexane, chloroform, ethyl acetate, methanol, and water were macerated with 10 g of powdered leaves, stems, and entire plant material (just the aerial part) by Soxhlet extraction at a certain temperature. In order to obtain a sticky mass, moisture at ambient temperature was removed before the final yield.

PHYTOCHEMICAL ANALYSIS:

TESTS FOR ALKALOIDS:

a) Dragendorff's reagent test: Weigh approximately 0.2 gm of each plant extract, add 2% sulphuric acid, and warm for an additional two minutes. The mixture was then filtered into a different test tube, some drops of Dragendorff's reagent were mixed in, and the presence of alkaloids was detected by looking for an orange red ppt.

b) The Mayer's test involved treating 1 ml of plant samples with 1 ml of the potassium mercuric iodide solution in Mayer's reagent. Alkaloids are confirmed by a precipitate with a cream or pasty yellow appearance.

TESTS FOR FLAVONOIDS:

When plant samples are combined with a few drops of ferric chloride, a dark green colour develops, indicating the presence of flavonoids.

The presence of flavonoids is confirmed by the alkaline reagent test in which test solution

exhibits a bright yellow colour when treated with sodium hydroxide solution. This colour disappeared after a few drops of diluted hydrochloric acid were added.

TESTS FOR SAPONINS:

a) The foam test produced persistent foam when plant extract and water were shaken quickly. This suggests that saponins are present.

TESTS FOR TANNINS AND PHENOLIC COMPOUNDS:

a) The ferric chloride test: 1 ml of plant samples were obtained, and 5% ferric chloride solution was added. The creation of a deep blue-black colour indicated the presence of phenolic compounds.

b) The acetic acid test: Add a few drops of acetic acid solution to 2- to 3-ml of extract; when red colour forms, tannins and phenols are present.

RESULTS AND DISCUSSION

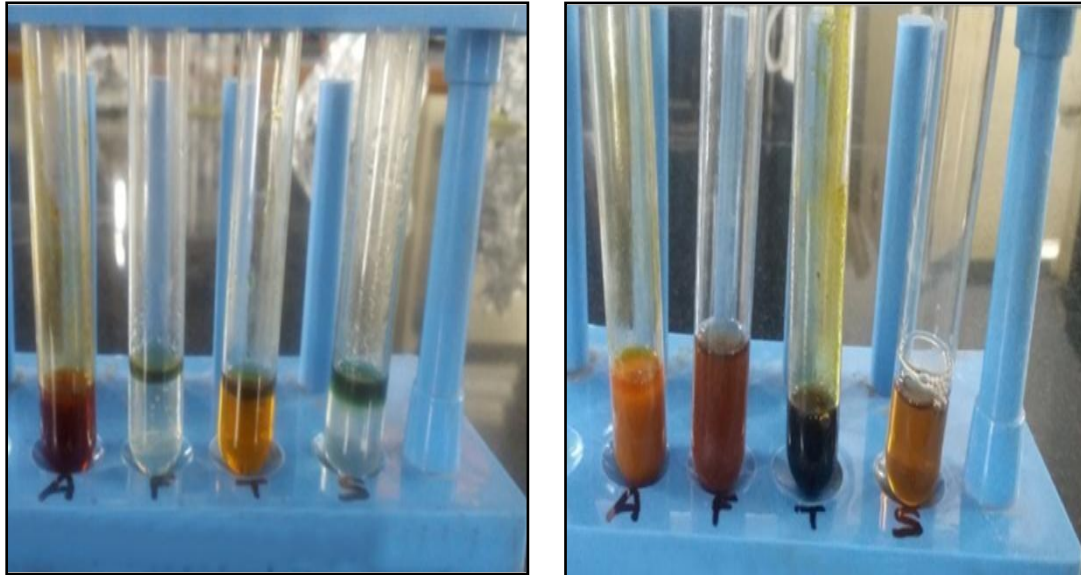
Ageratum conyzoides, plant samples, underwent preliminary screening to determine the presence of several bioactive components. The use of these plants as traditional medicines is supported by the discovery of bioactive components in them.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF VARIOUS EXTRACTS OF AGERATUM CONYZOIDES OBTAINED THROUGH SOXHLET EXTRACTION METHOD

Solvent	Phyto constituents	Alkaloids	Flavonoids	Saponins	Tannins & phenolic compounds
n- Hexane	S16	+	+	+	+
	S21	+	+	+	+
	S26	+	-	+	+
Ethylacetate	S17	+	+	+	+
	S22	+	+	+	-
	S27	+	+	+	+
Chloroform	S18	+	+	+	+
	S23	+	+	+	+
	S28	+	+	+	+
Methanol	S19	+	+	-	-
	S24	+	+	+	+
	S29	+	+	+	+
Water	S20	+	+	-	-
	S25	+	+	+	-
	S30	+	-	+	+

+ = Presence of constituents, - = Absense of constituents

Ageratum conyzoides stem and leaf are referred to as A.C.L. and A.C.S., respectively. Ageratum conyzoides whole plant extract (A.C.W.P. S 26 = Ageratum conyzoides Whole plant ext. S 16 = Ageratum conyzoides n- Hexane leaf ext. S 21 = Ageratum conyzoides leaf n- Hexane stem ext. S 18 represents Ageratum conyzoides chloroform leaf ext., S 23 represents Ageratum conyzoides chloroform stem ext., S 28 represents Ageratum conyzoides whole plant ext., S 17 represents Ageratum conyzoides ethylacetate leaf ext., S 22 represents Ageratum conyzoides ethylacetate stem ext., S 27 represents Ageratum conyzoides.



QUANTITATIVE ESTIMATION-

Ageratum conyzoides was likewise examined in the same way to separate all four phytochemicals. The total alkaloid content ranged from 0.099 g in the stem and 0.585 g in the leaf to 0.781 g in the entire plant portion. *Ageratum conyzoides* flavonoids were ultimately extracted from the leaf, stem, and root, totaling 0.025g, 1.371g, and *Ageratum conyzoides*. An entire plant weighed 1.114g. *Ageratum conyzoides* had leaves that had 1.51 g of saponins, stems that contained 2.741 g, and a whole plant that contained 3.149 g. *Ageratum conyzoides* leaves yielded 1.550 g of tannins, 3.760 g of tannins from the stem, and 1.805 g of tannins from the complete *Ageratum conyzoides* plant (Table 4.6).

TABLE 2: QUANTITATIVE ESTIMATION OF ALKALOIDS, FLAVONOIDS, SAPONINS AND TANNINS IN LEAVES, STEMS AND WHOLE PLANT OF AGERATUM CONYZOIDES

Phytoconstituents	Ageratum conyzoides		
	Leaves	Stem	Whole plant
Alkaloids	0.596 gm	0.000gm	0.792gm
Flavonoids	0.036gm	1.382gm	1.125gm
Saponins	1.62gm	2.752gm	3.150gm
Tannins	1.561gm	3.771gm	1.816gm

CONCLUSION

Ageratum conyzoides was used in the current work to screen significant and beneficial phytochemicals and evaluate their pharmacological effects. By selecting the right solvents, plant material was extracted in order to separate the phytochemicals from the plant. To analyse the phytoconstituents in the plant sample, extraction was carried out using the Soxhlet method with five solvents ranging from non-polar to polar, namely n- Hexane, chloroform, ethylacetate, methanol, and water. The maximum amount of dry yield (19.55%) in the Verbesina encelioides extract showed the presence of phytochemicals that extract out in polar solvents, and the lowest amount (0.14%) was found stem N-hexane extract, clearly demonstrating the affinity of phytoconstituents present in the plant samples with polar solvents as compared to non-polar solvents. The Soxhlet extraction method was used to screen Ageratum conyzoides, and similar findings were achieved. The aqueous leaf extract (12.55%) produced the highest dry yield, whereas the n-hexane stem extract (0.25%) produced the lowest. This brings up the fact that the phytochemicals found in Ageratum conyzoides and Verbesina encelioides are more soluble in polar solvents than non-polar solvents. There is a considerable variation between the percentages of the extracted mass, according to the results of the extraction procedure for both plants. Thus, the prevalence of bioactive metabolites derived from plants and their importance in medicine are undeniable. Scientists have become

extremely enthusiastic and interested as a result, and there are now unheard-of potential in the fields of biotechnology and the quickly growing natural product businesses. Pharmacological screening of plant extracts followed by a bioassay-guided fraction that isolates pure components is one of the effective methods for the discovery of therapeutic compounds from higher plants. However, one of the obstacles of herbal products is standardisation before making it a commercially useable product in order to maintain a stable quality and therapeutic efficacy. In situations when the dosage is critical, poisonous plants with purported synergistic activity should definitely not be used. Care must be exercised with potent plants until the nature of the interaction is understood and extracts are standardised to incorporate what is known, albeit there is no use in discounting the wisdom of experience! It does place attention on the special characteristics of herbal remedies and can provide valuable lessons about how to efficiently and with few side effects treat disease.

REFERENCES

1. Krishnaraju, A.V., Rao, T.V.N., Sundararaju, D. (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay, *Int J Appl Sci Eng* **3(2)**: 125-34.
2. Kubinyi, H., Folkers, G., Martin, Y. C.(1998) *3D QSAR in Drug Design: Volume 2: Ligand-Protein Interactions and Molecular Similarity (Vol. 2)*. Springer Science and Business Media.
3. Kumar, K., Malik, S., Demeria, D. (2002) Treatment of chronic pain with spinal cord stimulation versus alternative therapies: cost-effectiveness analysis, *Neurosurgery* **51(1)**: 106-116.
4. Kumarasamy, Y., Cox, P. J., Jaspars, M., Nahar, L., Sarker, S.D. (2002) Screening seeds of Scottish plants for antibacterial activity, *J Ethnopharmacol*, **83(1)**: 73-77.
5. Kurz, W.G.W., Constabel, F. (1998) Production of secondary metabolites. In: *Agricultural Biotechnology*. Altman A (Ed.). Marcel Dekker Inc, New York, USA, pp 183-224.
6. Lai, P.K., Roy, J. (2004) Antimicrobial and chemo preventive properties of herbs and spices, *Curr Med Chem* **11 (11)**: 1451–60.

7. Lasker, J.M., Huang, M.T., Conney, A.H. (1984) In vitro and in vivo activation of oxidative drug metabolism by flavonoids., *J Pharmacol Exp Ther* **229(1)**: 162-170.
8. Lauk, L., Lo bam, M., Rapisarda, A., Blandino, G. (2003) Antibacterial activity of *Calendula officinalis* against anaerobic and facultative aerobic bacteria, *Phyto Res* **17(6)**: 599-604.
9. Mulabagal, V., Tsay, H.S. (2004) Plant cell cultures-an alternative and efficient source for the production of biologically important secondary metabolites, *Int J App Sci En.* **2(1)**: 29-48.
10. Muley, B.P., Khadabadi, S.S., Banarase, N.B. (2009) Phytochemical constituents and pharmacological activities of *Calendula officinalis* Linn (Asteraceae): A review, *Trop J Pharm Res* **8**: 454-465.
11. Nadkarni, A.K. 1982. *The Indian Materia Medica*. Vol11. Popular Prakashan Pvt. Ltd, Bombay, India.
12. Nair, R., Chanda, S. (2007) Antibacterial activities of some medicinal plants of the western region of India, *Turk J Biol* **31(4)**:231-236.
13. Nair, R., Kalariya, T., Sumitra, C. (2005) Antibacterial activity of some selected Indian medicinal flora, *Turk J Biol* 29: 41-7.
14. Napier, L.D., Stanfill, A., Yoshishige, D.A., Jackson, K.E., Barron, B.A., Caffrey, J.L. (1998) Autonomic control of heart rate in dogs treated chronically with morphine, *Ame J Physiol Heart Circ Physiol***275(6)**: H2199- H2210.