PHYTOCHEMICAL AND BIOLOGICAL STUDIES ON *AGERATUM CONYZOIDES* L.

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***Abstract*– The ultrasonic assisted extraction method is more efficient as compared to normal conventional methods. The current study was aimed to perform ultrasonic assisted extraction of *Ageratum conyzoides*. Natural solvents such as n-hexane, dichloromethane, methanol and ethyl acetate were utilized for the purpose. Phytochemical studies indicated terpenoids, alkaloids, sterols, flavonoids, cardiac glycosides, saponins and coumarins as major constituents. The biological potential of various fractions was also determined in different extracts. In antibacterial and antifungal assays, a reasonable activity was obtained. All the extracts were also screened for Leishmanicidal and cytotoxic potential against Leishmania major promastigotes and 3T3 cancer cells, respectively. The methanol and ethyl acetate extracts showed significant antileishmanial activities i.e. IC_{50} 1.61 ± 0.50µg/ml and IC_{50} 15.21 ± 0.61µg/ml, respectively.**

***Keyword*– Ultrasonic assisted extraction (UAE), *Ageratum conyzoides*, Phytochemicals, Biological studies**

I. INTRODUCTION

The treatment of different health problems and as a source of inspiration for new drugs, the plants have been used for a very long time (Shakoor *et al*., 2018). It is reported that mostly people in rising country are dependent upon the conventional herbal medicines for their healthiness harms (Khan *et al*., 2018).

Ageratum (Asteraceae) consists of approximately 30 species, but only a few species have been phytochemically investigated. The plants are widespread in hot and humid areas of the world. The majority of these plants are present in Southeast Asia, West Africa, South America, South Eastern North America and Central America (Okunade, 2002; Bamidele *et al.*, 2010). In Pakistan, these plants exist in Islamabad, Rawalpindi and Changa Manga forest near Lahore (Zafar *et al.*, 2006).

The extraction of secondary metabolites from plants by using different organic solvents through conventional extraction methods is time consuming and less efficient (Parkash *et al*., 2017). The ultrasonic assisted extraction method is widely used for the extraction of phenolic constituents (Tiwari *et al*., 2010). The importance of this method over other conventional methods is due to its mechanical effects by increasing the solvent penetration into the products and is used for the extraction of thermally unstable compounds (Wu *et al*., 2001).

The plants of this genus contain a wide range of bioactive chemical constituents, including alkaloids (Wiedenfeld and Roder, 1991), cumarins (Desai *et al*., 1973), flavonoids (Adesogan and Okunade, 1979), chromenes (Pham *et al*; 1976), benzofurans (Pari *et al*., 1998), sterols (Dubey *et al*., 1989; Kamboj and Saluja 2011) and terpenoids (Horng *et al*., 1976). Various plant extracts of these plants have been found to have pharmacological and insecticidal potentials (Achola *et al*., 1994; Kamboj and Saluja, 2008). *A. conyzoides* is traditionally used in folk medicine in various parts of the world, including Africa, Asia and South America (Singh *et al*., 2013). The plant extract is found to have cardiovascular depressant, antispasmodic, antioxidant, haemostatic, anti-inflammatory, antispasmodic, antiasthmatic and insecticidal activities and is used as a remedy in the treatment of wounds and bacterial infections (Amal *et al*., 2010; Gonzalez *et al.*, 1991; Nour *et al.*, 2010; Panni and Bakht, 2018; Trease and Evans, 1989;). *A. conyzoides* has larvicidal and growth inhibitory activity and possess analgesic and anti-inflammatory activities with no apparent hepatotoxicity (Arya *et al.*, 2011; Kamboj and Saluja 2011; Magalhaes *et al*., 1997). The root of the plant found useful in fever and used as anti-helminthic and anti-dysenteric (Moura *et al.*, 2005).

Taking into consideration the immense importance of these plants, the present work was therefore carried out.

II. METHODS

A. Plant material

The *A. conyzoides* plant was collected from Islamabad, Pakistan in March- April, 2014. The species was identified by Chairman Department of Botany, Quaid e Azam University and a specimen voucher No. 656 has been placed at herbarium for future reference.

B. Extraction and fractionation

The plant material (5Kg) was washed with clean water, chopped and dried under shadow in air. The powdered plant material was extracted with natural solvents including hexane, dichloromethane, methanol and ethyl acetate. The extract of the plant was added in a gradient manner to two liters of solvent, resulting residue was subjected mechanically to stirring for 24 hours with ultrasonic waves for efficient extraction. The extract was decanted and the solvent was dried under vacuum. The different extracts were used for the determination of phytochemical analysis and biological potential.

Table 1. Phytochemical screening of four different extract of *A. conyzoides*

Phytochemicals	Hexane	DCM	EtOAc.	MeOH
Alkaloids	+	+	+	+
Tannins	-	-	-	-
Saponins	+	+	+	+
Sterols	+	+	+	+
Flavonoids	-	+	+	+
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	+	-
Coumarins	-	+	+	-
Anthocyanidins	-	-	-	-
Anthraquinones	-	-	-	-
Phlobatanins	-	-	-	-

C. Phytochemical Study

The phytochemical study of all the extracts was carried out by using the standard procedures (Basu and Kirtikar 1991) and results are summarized in Table 1.

Alkaloids

Alkaloids presence was determined by mixing 200 mg of each fraction with 2% H_2SO_4 solution and heated for two minutes. The mixture was filtered and few drops of the reagent were mixed. The color of the mixture was changed to orange. The test confirmed that alkaloids were present in all the extracts.

Tannins

In order investigate Tannins in each fraction, about 500 mg each fraction was added to test tube containing 10 ml of deionized water and was boiled. The mixture was filtered and few drops of 0.1 % FeCl3 solution was added. No change in the color of substrate was observed which indicated the absence of tannins.

Saponins

The saponins were confirmed by dissolving 500 mg of each fraction in boiling H_2O_2 in a flask. The mixture was permissible to chill and be stimulated. The continuous foam formation confirmed that saponins are present in plant extract.

Sterols

The extract of Ageratum conyzoids material was added to test tube containing 1 mL of H_2SO_4 solution. On addition to H_2SO_4 the red colored appeared which indicated the presence of sterol in extract.

Flavonoids

To confirm the flavonoids in the plant extract, 200 mg of each fraction was liquefied in NaOH solution and after that hydrochloric acid was mixed. The change of fair-haired color to without colorless verified the occurrence of flavonoids.

Terpenoids

The terpenoids in fraction was confirmed by addition of 0.5g each of plant extract and fractions to 2 ml chloroform followed by addition of 3 mL of concentrated H_2SO_4. The appearance of reddish brown color confirmed the presence of terpenoids.

Cardiac glycosides

Determination of cardiac glycosides, 500 mg of fraction was added to flask containing 5 mL of water followed by addition of 2 mL of glacial acetic acid, one drop of $FeCl_3$ and 1 mL of concentrated H_2SO_4. The appearance of brown ring at the border established the presence of *Cardiac glycosides.*

Coumarins

About 0.5 g each of plant extract and fraction was added to the flask and enclosed with 1M NaOH solution soaked filter paper. The flask was immersed in hot hose for several min. After few min later filter paper was detached and was subjected to UV light. The appearance of yellow fluorescence justified the occurrence.

Anthocyanidins

In this test, 1 ml of HCl was added to 500 mg of each fraction was mixed with sulfuric acid. However, on addition of HCl no color changed was observed which confirm the absence of anthocyanidins.

Anthraquinones

500 mg of each fraction was added to 10 percent HCl and was boiled for few minutes. The extract and each corresponding fraction were filtered and cooled. Chloroform and several go down of 10 percent of ammonia was added in same volume to the filtrate and was heated. No color change was observed which indicated the absence of anthraquinones.

Phlobatanins

500 mg of each fraction was mixed in purify H_2O and clean. The scum was heated with 2 percent HCl solution. No significant change occurred which showed the absence.

D. Biological studies

All the extracts were subjected to check their biological potentials against various pathogens.

Antibacterial Activity

The antibacterial potential was determined by preparing samples of the corresponding extract (1 mg) in DMSO (1 ml) (Alzoreky and Nakahara, 2003). In this method (DMSO was used as negative control while both the antibiotics were used as positive control. Then by dissolving nutrient broth (0.4 g) in distilled water (50 ml), the nutrient broth medium was prepared. At pH 7, the medium was autoclaved at 121°C.

For bacterial growth, the nutrient agar intermediate was equipped by solving of 2.3 g nutrient agar in distill H_2O_2 (100 ml) having pH 7. Four different strains of bacterial (2 gram positive and 2 gram negative) were utilized viz; Streptococcus aureus Bacillus, subtilis (ATCC 6058) (ATCC 6537), *Klebsiella pneumonia* (ATCC 4351) and *Pseudomonas aeruginosa* (ATCC 7221) in medium at 4ºC. The selected bacterial strain of old cultures in nutrient broth was mixed with normal saline solution until they matched a McFarland turbidity standard. Results are summarized in Fig. 1.

Fungal inhibition assay

Fungal inhibition assay was dogged according to standard protocol of Koneman *et al*. (1988). Two different fungal strains including *Helimenthosporium sativum* and *Fusarium moniliforme* were maintain at 4ºC in SDA medium. All the test tubes of the assay were

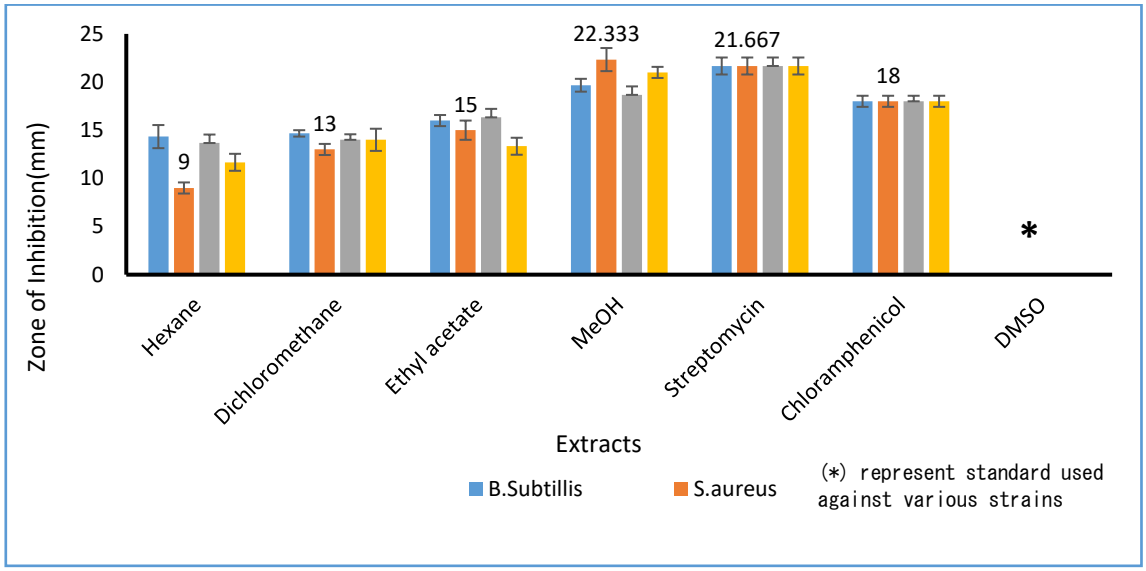

Figure 1: Antibacterial Activity of *A. conyzoids.*

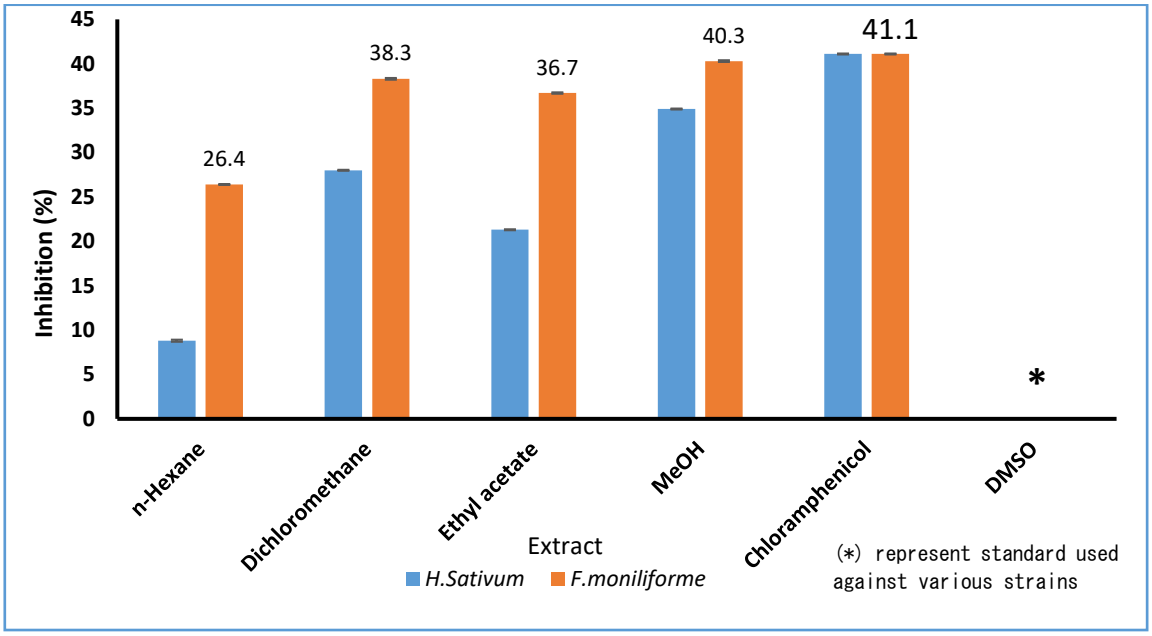

Figure 2: Antifungal activity of *A. conyzoids.*

retained for 7 days in incubator at 28°C. At the end % Inhibition of fugal growth was measured by following formula:

$$\%\text{Inhibition of fugal growth} = 100 - \frac{\text{Growth in test}}{\text{Growth in control}}100$$

Results are summarized in Fig. 2.

Antileshmanial Activity

Habtemariam (2003) method was used for determination of antileishmanial activity using 96 well plates at 21-22ºC. Cell viability was checked with the help of micro-scope. All assays were run in duplicate.

% Inhibition was calculated by following formula;

$$\%\text{Inhibition} = \frac{\text{OD of control} - \text{OD of treated}}{\text{OD of control}}100$$

where OD means Optical density. Results are summarized in Fig. 3.

Cytotoxic Activity

Yeskaliyeva *et al.* (2006) protocol was used for determination of Cytotoxicity of all the fractions through MTT colorimetric method (Mosmann 1983) using 3T3 cells (mouse fibroblasts). The degree of MTT decrease to formazan dye with in cells was deliberated by dimension of the OD at 540 nm through ELISA. Cytotoxicity was renowned as the attentiveness cause 50 % expansion reserve. Results are summarized in Fig. 4.

III. RESULTS

Phytochemical Screening

In this study, phytochemical screening of four different extract of *A. conyzoides* was carried out which provided incentive for proper evaluation of this plant for its medicinal use. According to the results (Table 1) tannins, phlobatanins, anthraquinones, anthocyanidins were

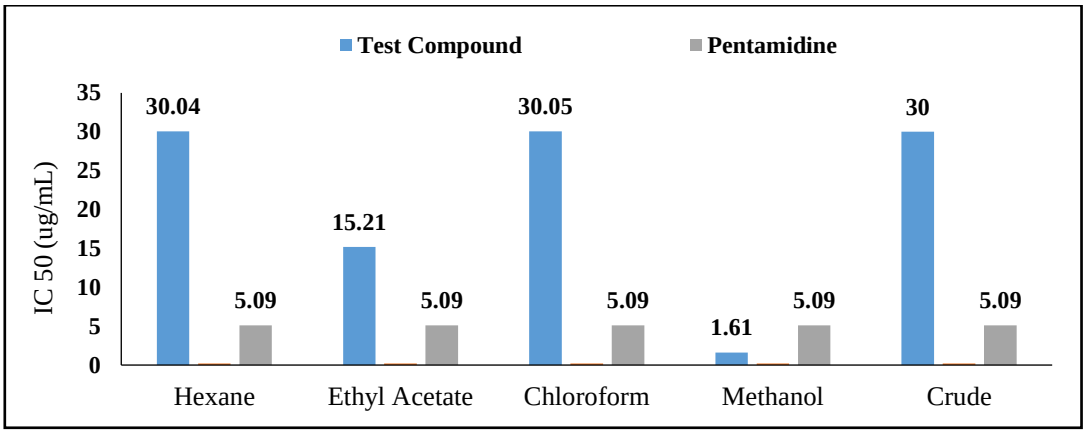

Figure 3: Leishmanicidal Activity of *A. conyzoids*

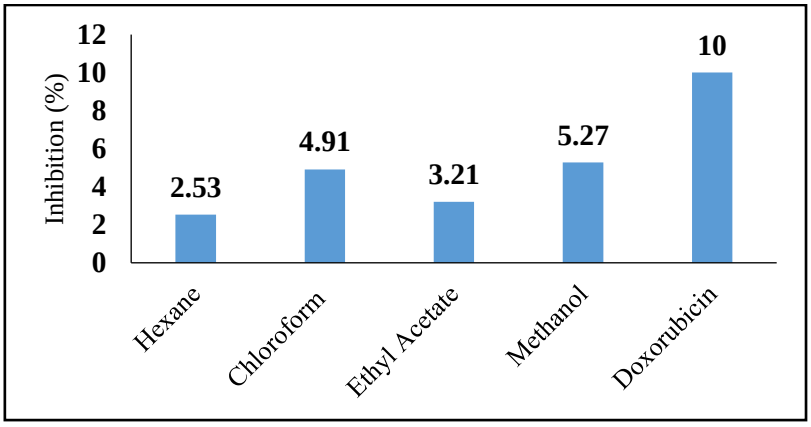

Figure 4: Cytotoxic Activity of *A. conyzoids*

absent in all extracts. While flavonoids, cardiac glycosides, sterols, saponins, terpenoids and alkaloids were found in all extracts except for flavonoids which were absent in hexane extract and cardiac glycosides in methanol extract. Coumarins were also absent in hexane and methanol extract but present in dichloromethane and ethyl acetate extract.

Antibacterial Activity

Bioassay for four different extracts of *A. conyzoides* was conducted to compare the relative activity of these extracts (Fig. 1). These results showed clearly that activity of MeOH extract was highest against all the four strains of bacteria and was also comparable to standard drug used. The MIC (minimum inhibitory concentration) of the extract was investigated by intensity to a variety of attentiveness based on the worldwide consommé intensity method. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC (minimum bactericidal concentration). The activity of MeOH extract was greater than n-hexane, dichloromethane and ethyl acetate extracts whereas activity of n-hexane extract was lowest. So from these results it can be concluded that MeOH extract is more antibiotic in comparison to n-hexane and other portions.

Antifungal Activity

Results of antifungal activity (Fig. 2) showed highest activity of MeOH extract against both strains. Whereas activity of dichloromethane and ethyl acetate was also good and comparable to standard drug against *Fusarium moniliforme*. These results indicated that polar compounds extracted by polar solvents were more antifungal in nature. So the extract of highest polarity (MeOH extract) showed highest activity and extract of lowest polarity (n-hexane extract) showed the lowest activity.

Antileishmanial Activity

Antileishmanial efficacies of the extracts were evaluated. Pentamidine was used as standard leishmanicidal drug. The methanolic extract showed potent leishmanicidal activity (IC50 5.61 ± 0.5 µg/mL) and ethyl acetate showed significant activity (IC50 23.21 ± 0.61 µg/mL). Other fractions showed less activity. The data (Fig. 3) revealed that the polar extracts MeOH and ethyl acetate could serve as an alternative agent for the control of leishmania disease.

Cytotoxicity

The extracts were screened for cytotoxicity (Fig. 4) against 3T3 fibroblast cells. All the extracts were inactive, compared with the standard anticancer drug, doxorubicin (IC50 10 µmol). The low cytotoxicity values of the tested extracts reflect non-toxic nature of this plant which is important to use this plant in further studies for the drug development against different disease.

IV. CONCLUSIONS

The results indicated the terpenoids, alkaloids, coumarins, sterols, flavonoids, cardiac glycosides and saponins as major constituents of plant. A reasonable antibacterial

and antifungal potential was exhibited. The low percent inhibition or stimulation of the tested extracts in cytotoxic assay was found.

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