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EVALUATION OF ANTIMICROBIAL, PHYTOCHEMICALS, TOTAL PHENOLIC AND FLAVONOID CONTENTS OF *VERBESINA ENCELIOIDES*- A LESSER KNOWN HERB OF FAMILY *ASTERACEAE*.

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Abstract *– Verbesina encelioides is an important medicinal herb belonging to the family Asteraceae (Compositae). It is a drought tolerant, have aggressive and very dominant growth abilities. Medicinal plants are rich sources of biologically active compounds which posses traditional medicinal treatment for various afflictions. Flavonoids are the phytoconstituents that provide protection against ultraviolet radiations and phenolic compounds, have biological and pharmacological properties. The present study was carried out to evaluate the methanolic extracts of the aerial vegetative parts of Verbesina encelioides; and studied for its purported antibacterial activity against gram positive and gram negative bacterial strains by disc diffusion method, phytochemical analysis and total phenolic and flavonoid contents were evaluated.*

Keywords: *Verbesina encelioides*, Antimicobial activity, Asteraceae, Phytoconstituents, Plant extracts.

I. INTRODUCTION

Verbesina encelioides, commonly it is known as wild sunflower, golden Crownbeard, Yellow top etc. It is a drought tolerant plant and is competitive and in its growth abilities. It belongs to the family Asteraceae (Earlier the Compositae family), is an erect, annual (**Wagner, 1990**) usually have height near about 1 - 5 feet (**Ball *et al*., 1951, Robbins *et al*., 1951; Parker 1972)** is also a wild plant and can tolerate huge range of climatic conditions. **(Kaul and Mangal, 1987**). Different parts of this plant such as the leaf, stem and flower are useful and important in pharmaceutical industries. This plant is purported to have antimicrobial, antiviral, anti-tumor, hypoglycaemic (Low BP) and anti-implantation efficacies (**Jain *et al*., 1988; 2007).** Asteraceae (Compositeae) is commonly referred to as the aster, daisy or sunflower family. It is the largest family of all the flowering plants and the genera are likely predicted about 1600 and the species probably 23000. They are distributed over most of the earth and in almost in all habitats. The greater proportion is herbaceous, although about 2 percent are trees or shrubs.

Plants usually seen to have taproots but fibrous root can also be present. Stem is generally erect, but sometimes it can be prostrate to climber. Some species have underground stems in the form of caudices or rhizomes. The leaves and the stems very often contain secretary canals with resin ducts or latex (very common among the subfamily Cichorioideae). The leaves are generally alternate, and can also be opposite or whorled. Leaves may be simple, but generally deeply lobed. The margins can be entire to dentate. Members of family Asteraceae are very common in open and dry environments. Many members of the Asteraceae are pollinated with the help of insects, which explains their value in attracting beneficial insects, but anemophily is also present in some genera (e.g. *Ambrosia* and *Artemisia*). There are many apomictic species in the family. They produce iso or chlorogenic acid, sesquiterpene lactones, pentacyclic triterpene alcohols, various alkaloids, acetylenes and tannins. (**Heywood *et al*., 2007)** The present study is carried out to evaluate the methanolic extract of the mature aerial vegetative part of *V.encelioides* and studied for its purported antibacterial activity against some gram positive and gram negative bacterial strains by disc diffusion method, phytochemical analysis and total phenolic and flavonoid content were also evaluated by standard methods.

II. METHODOLOGY

Plant material collection

Healthy plants of *V. enceliodes* were collected from road side in the month of May, 2016 from the area of Block Bawani Khera, District Bhiwani, and Haryana. The authenticity of the plant was confirmed from the Department

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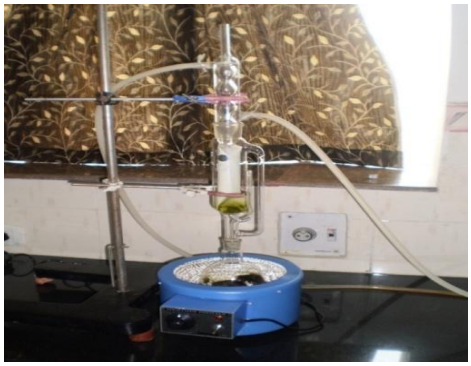
of Botany, M.D.U Rohtak . Then the aerial vegetative parts of the plants were washed under running tap water then with the DW and then dried under shade until the weight was constant.

Collection of *V. enceliodes from road side*

Dried under the shade



Extraction in Soxhlet Appratus

Fig 1.

Preparation of plant extract

After drying the aerial vegetative parts of the plant under the shade plant parts were powdered in electrical grinder. Powder was extracted in methanol by Soxhlet apparatus for relevant periods of time. The extract was stored at 4 º for further use.

Preparation of innoculum

Active cultures to carry out experiments were prepared by transferring a loopful of culture from the stock to test tubes of nutrient broth for bacteria and incubated for 24 hours at 37°C. The cultures were furthur diluted with fresh Nutrient Broth (Himedia) to achieve optical densities corresponding to 0.5 McFarlandstand (which is equal to 1.5 x 10^8CFU/ml).

Antibacterial screening

The methanolic extract of aerial vegetative parts of *V.enceliodes* was screened out against gram positive and gram negative bacterial strains. The test strains were procured from IMTECH Chandigarh. The antibacterial activities of the plant extract were evaluated by Disc diffusion method (**Baur, 1996**). Nutrient agar (Hi-media) was used for the screening. Four different concentrations of plant extract were prepared. The discs (6mm) of Whatman filter paper No 3 was used and plates were incubated at 37°C for 24 hours. Broad spectrum antibiotic Ampicillin was used as positive control and methanol was used as negative control. The diameter of inhibition zone (mm) was measured with the help of (Antibiotic Zone Scale, HI Media).

Phytochemical analysis:

Qualitative phytochemical screening was carried out for the presence of various phytochemicals like phenols, saponins, glycosids flavonoids, alkaloids and tannins etc as per the standard methods. (**Khandelwal, 2006; Kokate, 1999**).

Quantitative test for flavonoid and phenol

Quantitative screening for phenols and flavonoids were carried out by Folin-Ciocalteu reagent method and Aluminium chloride colorimetric method respectively (**Aiyegroro and Okoh, 2010**).

III. RESULTS AND DISCUSSION

Antibacterial screening

Methanolic extract of *V.enceliodes* was investigated to evaluate their antibacterial activity against gram positive and gram negative bacteria using disc diffusion method. Evaluation of antibacterial activity of methanolic extract of *V.enceliodes* was recorded in table no-1, graphically represented in Fig. 2 and illustrated in Fig. 3. The results revealed that the methanolic extract of *V.enceliodes* was potentially effective in suppressing microbial growth of both the type of bacteria strains (gram positive and gram negative) in all the four concentrations.

From the results of antibacterial activity of the of *V.enceliodes* it can be suggested that *E-coli* was the most resistant strain with (5.3mm) Zone of inhibition(ZOI) while the *Chromobacterium violaceum* was the most susceptible strain(11.2mm) ZOI.

Table No. 1

Extract	**Zone of Inhibiton(mm)**			
Methanol	**Concentration**	**Staphylococcus aureus (MTCC -96)**	***Chromobacterium violaceum* (MTCC-2656)**	***E-Coli* (MTCC-443)**
	100mg/ml	10.7±0.25	11.2±0.20	10.4±0.02
	50mg/ml	9.3±0.36	10.1±0.15	9.2±0.07
	25mg/ml	7.0±0.05	9.1±0.05	6.2±0.22
	12.5mg/ml	6.3±0.05	8.9±0.05	5.3±0.19
	Positive control	10.0±0.01	10.1±0.03	13.04±0.23
(All values are replicate. (n=3) Positive control-Ampicillin Negative control-Methanol				

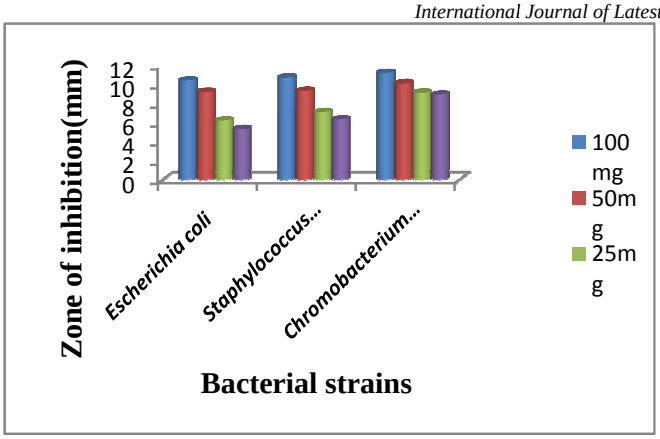
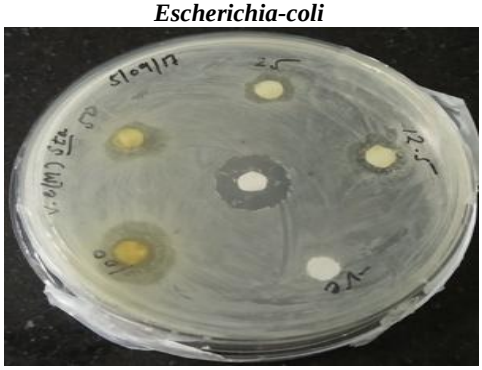
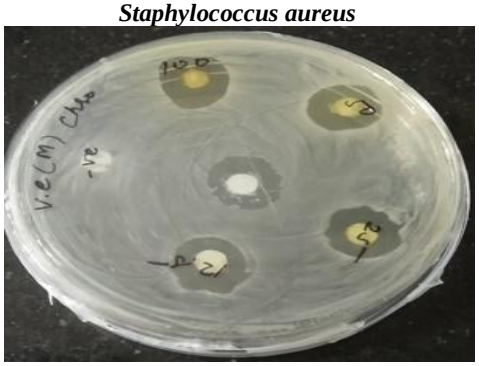


Fig.2. Screening of antibacterial activities of methanolic extract of *V.enceliodes*

Escherichia-coli



Staphylococcus aureus



Chromobacterium violaceum

Fig. 3

Phytochemical analysis of *V.enceliodes*

Preliminary phytochemical analysis was carried out for screening secondary metabolites which are responsible for a number of biological properties and for curing various ailments. Crude methanolic extract indicated the presence of various phytoconstituents as recorded in table no-2

Table No-2

Solvents	Glycosides	Saponins	Terpenoids	Steroids	Alkaloids	Phenol	Flavonoid
Methanol	+	-	-	-	+	+	+
Acetone	+	+	+	+	+	+	+
Chloroform	+	-	-	+	-	+	+
Petroleum ether	-	-	+	+	-	+	+

Note: Present (+), Absent (+)

Total phenolic and flavonoid content

The total phenolic content of methonolic extract of *V.enceliodes* was calculated from the calibration curve was 21.01 gallic acid equivalents/g and the total flavonoid contents was 68.11 Quercetin equevalents/g.

Table No- 3.

Total phenolic and flavonoids content of methanolic extract of *V.enceliodes*

Total phenolic content[1]	21.01±0.05
Total flavonoid content[2]	68.11±0.02

1. mg gallic acid equivalents(GAE)/g
2. mg quercetin equevalents/g.

All values are mean of three biological replicates (n=3)

CONCLUSION

Hence from the present study, it may be concluded that the phytochemicals play a definitive role in the biological activities of the plants. Plants have great potential as antimicrobial compounds against microorganisms and they can be used in the treatment of curing various types of ailments**.** Screening of plants for antimicrobial compounds and identifying the agents is the need of hour.

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