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A COMPARATIVE EVALUATION OF ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF LEAVES & FLOWERS OF *MIKANIA MICRANTHA* KUNTH FROM PASCHIM MEDINIPUR, WEST BENGAL, INDIA

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ABSTRACT: *Mikania micrantha* Kunth is a noxious weed of Asteraceae, which is the biggest enemy of biodiversity right now, but we cannot disregard the plant's therapeutic potential**.** This study evaluated the antioxidant and antibacterial properties of leaves and flowers from *Mikania micrantha* Kunth. Antibacterial screening was done by agar well diffusion method using Gentamicin (4mg/ml stock) as a standard antibiotic. The active extract's minimum inhibitory concentration (MIC) was determined using the broth dilution method. The antioxidant activity was evaluated by DPPH method using Ascorbic acid as standard. The results showed that leaf and flower is antioxidant property correlates with their total phenol and flavonoid content. The flower extract exhibited highest antioxidant properties with IC_{50} value of 60.44 µg/ml. Leaf extract showed very strong antibacterial properties against wound infection-causing bacteria like *Staphylococcus aureus* MTCC 87 and *Streptococcus pyogens* MTCC 437. Minimum inhibitory concentration (MIC) of the active extract against *Staphylococcus aureus* MTCC 87, *Streptococcus pyogens* MTCC 437 was 400µg/ml and 800µg/ml, respectively. The result of this study indicates that the flower of *Mikania micrantha* contains high phenols and flavonoids which is responsible for their strong antioxidant property. So, the flower extract has the potential to be used as a natural antioxidant. The result of antibacterial study also scientifically validates the use of *Mikania micrantha* leaf paste for cut and wounds in traditional medicine.

INTRODUCTION: Plants have been used for therapeutic purposes since primordial times [1]. Plant-based medicine is considered safer as it has fewer side effects than synthetic drugs [2]. According to WHO, about 80% of people worldwide use plants as medication for the treatment of common illnesses [3].

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Different phytochemicals present in plants are responsible for their therapeutic potential. The phytochemical composition varies in different parts of the plant, so it is important to know which part of the plant has the pharmacological potential.

So, research about the therapeutic potential of plant parts is required to use plant parts as raw material for producing new drugs. *Mikania micrantha* Kunth, also known as “Taru-lota” in Paschim Medinipur, is a climbing perennial weed of Asteraceae that was introduced in India in the 1940s [4]. The plant is native to Central and South America [5].

Although the plant has been listed as one of the World's 100 worst invasive alien species [6], its medicinal importance cannot be ignored. This weed is famous in many parts of the World for wound dressing and healing of sores [7-9]. In Purba and Paschim Medinipur district of West Bengal, the leaves of *Mikania* are popularly used to stop bleeding immediately from recent cuts and wounds [10-11]. Although maximum research has been done on the leaves of this plant, work on its flowers is still unexplored. So, this investigation was conducted to explore the antioxidant and antimicrobial potential of leaves and flowers from *Mikania micrantha* kunth.

METHODOLOGY:

Sample Preparation: The fresh leaves and flowers of *Mikania micrantha* were collected from various part of Paschim Medinipur. The herb was identified with the help of standard literature [12]. The collected samples were washed thoroughly with tap water until all the dirt was removed. The leaves and flowers were then air-dried for 3-4 days and powdered with the help of a grinding machine. The powdered samples were tightly packed in a zipper bag and kept at 4ºC for future use.

Extraction: About 15 gm of dried powder was macerated with 150 ml of 95% ethanol for 3 days. The liquid extract was filtered with the help of Whatman No.1 filter paper. Re-maceration was carried out until the extract turned colorless. After many cycles of maceration, filtrate from each cycle was mixed, and the solvent was evaporated using rotary evaporator to obtain a semisolid ethanolic extract.

FIG. 1: *MIKANIA MICRANTHA* IN IT'S NATURAL HABITAT

Determination of Total Phenolic Content (TPC): Folin-Ciocalteu methods [13] were used to determine the total phenolic content, with slight modifications. About 1 ml of sample with concentration of 1 mg/ml was taken in a test tube and 1 ml of 7.5% Na_2CO_3 was added into it. After 8 minutes, 1.25 ml of Folin-Ciocalteu's reagent (1:1) was added into it and incubated for 30 minutes. The absorbance was taken at a wavelength of 760 nm using a UV-Vis spectrophotometer. The standard curve of galic acid was prepared with concentrations of 10, 30, 50, 70, 90 and 100 µg/ml. The total phenolic content was calculated in milligram galic acid equivalent per gram of sample (mg/g).

Determination of Total Flavonoid Content (TFC): Total flavonoid content was determined by aluminium chloride colorimetric method [13] with slight modifications. The mixture of 1 ml sample (1mg/ml), 4 ml water, 0.3 ml 5% NaNO3, 0.3 ml Alcl3 and 1 ml of 1M NaOH was taken in a volumetric flask. The final volume in the flask was made upto 10 ml with distilled water. Quercetin was used as a standard for the determination of total flavonoid. The absorbance was taken against blank at a wavelength of 510 nm using a UV-Vis spectrophotometer. The total flavonoid content was calculated in milligram quercetin equivalent per gram of sample (mg/g).

***In-vitro* Antioxidant Assay:** The antioxidant activity of the extract was evaluated using DPPH method [14] with slight modifications. A stock solution of the extract was prepared by dissolving 50 mg of the semisolid extract into 50 ml of 80% ethanol. Different concentrations (20, 40, 60, 80 and 100µg/ml) of the extract were prepared from the stock solution for this experiment. About 1 ml the extract was mixed with 3 ml of 0.16Mm DPPH and incubated for 30 minutes. Ascorbic acid, 80% ethanol was taken as positive control and negative control, respectively. The degree decolourisation of DPPH was measured by taking absorbance at 517 nm using a spectrophotometer. The absorbance of each test sample (Leaf, Flower and Ascorbic acid) was plotted and IC_{50} Value (concentration of test sample required to cause 50% inhibition) was calculated.

Antibacterial Assay: The agar well diffusion method[15] was used to determine the antibacterial activity of the ethanolic extract. About 15-20ml of molten Muller-Hilton agar was poured on glass petriplates of the same size and allowed to solidify.

A sterilized cork borer made Four wells into the Muller Hilton agar. Standardized inoculums of test organisms (*Staphylococcus aureus* MTCC 87, *Streptococcus pyogens* MTCC 442, and *Pseudomonas aeruginosa* MTCC 741) were uniformly spread on the surface of plates using sterile spreader. Semi-solid ethanolic extract was dissolved in 20% DMSO to prepare a stock solution of 10 mg/ml. About 40 µl and 80µl of the test sample were added into the well T1 and T2 from the stock solution (10mg/ml). Gentamicin (40µl from 4 mg/ml stock) was added into the well as a positive control, and 20% DMSO was used as negative control. The petri-plates were incubated in an incubator for 24 h at 37ºC.

After incubation, the plates were observed, and the zone of inhibition around the well was measured in mm. Minimum inhibitory concentration (MIC) was determined using broth dilution method [16]. The test sample and standard antibiotic was diluted in LB broth to get a final concentration of 12.5 mcg, 25 mcg, 50 mcg, 100 mcg, 200 mcg, 400 mcg, 800 mcg and 1600 mcg. 50 µl log phase bacterial culture (1×10^7 CFU/ml) was added to each experimental well. An uninoculated broth was kept as blank. The microplate was incubated at 37˚. OD_{600} was checked with a spectrophotometer keeping the uninoculated broth as blank. MIC point was interpreted as the lowest concentration of antibiotic and test sample at which there is no visible growth of bacteria (absence of turbidity).

RESULTS:

Extraction: The physical properties of the sample, like the consistency of extract, colour of extract and percentage of extract yield, are shown in **Table 1.**

Total Phenol and Flavonoid: The flower extract showed higher phenol and flavonoid content than the leaf extract, as shown in **Table 2.**

Antioxidant Assay: Flower extract showed higher antioxidant potential than leaf extract. The IC_{50} value of the flower extract was 60.44 µg/ml, and for Ascorbic acid, it was 44.40 µg/ml. The result is shown in **Table 3.**

Antibacterial Assay: The ethanolic leaf extract showed the highest antibacterial activity against *Staphylococcus aureus* MTCC 87 and moderate activity against *Streptococcus pyogens* MTCC 437. The flower extract failed to show any activity against test organisms. The result is displayed in **Table 4** and **Table 5.**

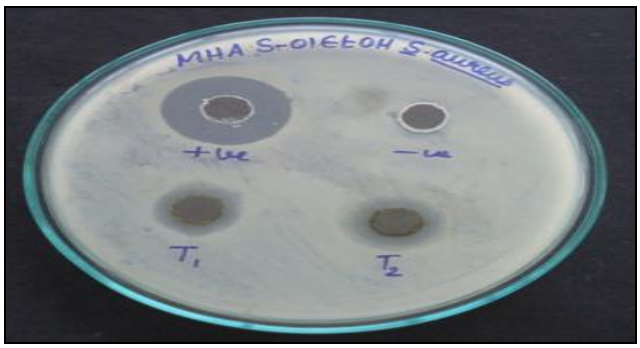

FIG. 2: ANTIBACTERIAL ACTIVITY SHOWN AGAINST *STAPHYLOCOCCUS AUREUS* BY ETHANOLIC LEAF EXTRACT OF *MIKANIA MICRANTHA*. +Ve – Positive Control (Gentamicin) -Ve – Negative Control (DMSO)

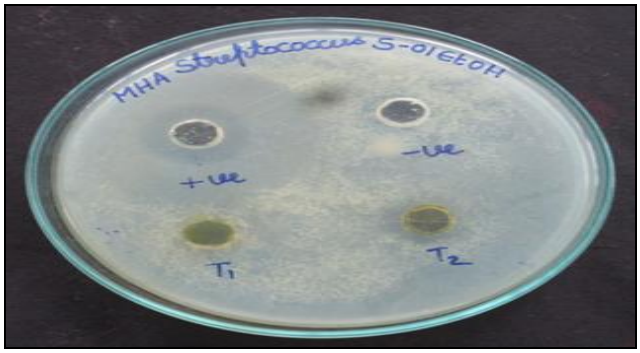

FIG. 3: ANTIBACTERIAL ACTIVITY SHOWN AGAINST *STREPTOCOCCUS PYOGENS* BY ETHANOLIC LEAF EXTRACT OF *MIKANIA MICRANTHA*. +Ve – Positive Control (Gentamicin) -Ve – Negative Control (DMSO)

TABLE 1: EXTRACTION YIELD (%) OF LEAF AND FLOWER EXTRACT FROM *MIKANIA MICRANTHA* KUNTH

Plant part	Solvent Extract	Consistency	Colour	Weight of extract (gm)	Yield (%)
Leaf	Ethanol	Semi solid	Dark Green	1.106	6.96%
Flower	Ethanol	Semi solid	Pale Greenish Yellow	0.783	5.22%

TABLE 2: TPC AND TFC OF LEAF AND FLOWER EXTRACT FROM *MIKANIA MICRANTHA* KUNTH ±: INDICATES STANDARD DEVIATION

Plant part	Total phenol (mg/g)	Total flavonoid (mg/g)
Leaf	97.12 ± 0.051	31.66 ± 0.501
Flower	115.51 ± 0.316	43.09 ± 0.080

TABLE 3: IC_{50} VALUE OF LEAF, FLOWER AND ASCORBIC ACID

Sample	Equation	IC_{50} Value (µg/ml)
Leaf	$y = 0.519x - 2.376$ $R^2 = 0.998$	100.91
Flower	$y = 0.796x + 1.888$ $R^2 = 0.998$	60.44
Ascorbic Acid	$y = 0.887x + 10.61$ $R^2 = 0.979$	44.40

TABLE 4: ANTIBACTERIAL POTENTIAL OF LEAF AND FLOWER EXTRACT FROM *MIKANIA MICRANTHA* KUNTH

Test Organism	ZOI (Leaf)		ZOI (Flower)		ZOI (Positive control)	ZOI (Negative control)
	T1	T2	T1	T2		
Staphylococcus aureus MTCC 87	15 mm	18 mm	-	-	22 mm	-
Streptococcus pyogens MTCC 442	-	14 mm	-	-	37 mm	-
Pseudomonas aeruginosa MTCC 741	-	-	-	-	30 mm	-

TABLE 5: MINIMUM INHIBITORY CONCENTRATION (MIC) OF TEST SAMPLE AND STANDARD ANTIBIOTIC

Test Organism	Minimum Inhibitory Concentration (MIC)	
	Leaf	Gentamicin
Staphylococcus aureus MTCC 87	400 µg/ml	100 µg/ml
Streptococcus pyogens MTCC 442	800 µg/ml	100 µg/ml

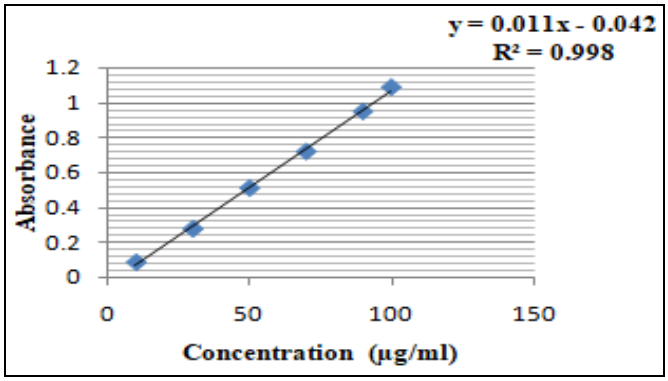

FIG. 4: GALLIC ACID CALIBRATION CURVE

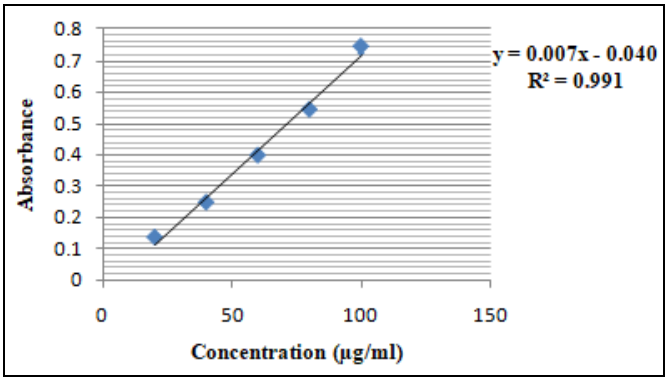

FIG. 5: QUERCETIN CALIBRATION CURVE

DISCUSSION: The plant has the potential to be used as a raw material in traditional remedies. The chemical constituents present in the plant are responsible for their pharmacological properties. *Mikania micrantha* is a wonderful plant utilized in herbal medicine for various purposes. The extraction of leaves and flowers using maceration by 95% ethyl alcohol showed very slight difference in yield (%) as shown in **Table 1.** The concentration of phenol and flavonoid in plant extract depends on the polarity of the solvent used for the extraction. Ethanol is reported to be the standardized solvent for extraction of *Mikania* [17]. The ethanolic extract from *Mikania* leaf showed less phenol and flavonoid content than the flowers. The result of TPC and TFC are correlated with the result of the DPPH antioxidant scavenging assay. Due to high phenol and flavonoid content the

flower extract exhibited high antioxidant potential with IC_{50} value of 60.44µg/ml. The phenolic compounds are those antioxidant agents which can operate as free radical terminators, metal chelator, lipoxygenase inhibitors, and free radical scavengers [18].

Flavonoids are also reported to have antioxidant properties [19] and may potentially overcome many disorders like cardiovascular diseases, infections, respiratory problems, and aging. The Agar well diffusion method determined the plant's antibacterial properties. The antibacterial activity against skin disease and wound infection-causing bacteria *Staphylococcus aureus* and *Streptococcus pyogens* was shown by ethanolic leaf extract, validating the traditional claim of using *Mikania* leaf paste for cuts and wounds.

Although flower extract exhibited higher phenol and flavonoid content, it failed to show any antibacterial activity, suggesting that other secondary metabolites are responsible for antibacterial property of *Mikania*. Sesquiterpene lactones from the leaves of *Mikania mcrantha* are reported to have antibacterial potential [20].

CONCLUSION: The result of this study shows that the phenolic and flavonoid content of the leaf and flower is correlated with their antioxidant properties. Flower extract can be used as a natural antioxidant as it showed IC_{50} value of 60.44µg/ml compared to IC_{50} value of 44.40µg/ml of standard ascorbic acid. The other part of this study also indicates that the leaf extract can be used topically to prevent skin disease and wound infection caused by *Staphylococcus aureus* and *Streptococcus pyogens*. Furthermore, *in-vivo* studies are required to introduce *Mikania micrantha* into modern-day's medicine.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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