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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ANALYSIS OF THE LEAF EXTRACTS OF *LAUNAEA PROCUMBENS* ROXB.

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ABSTRACT

The present study evaluate the phytochemical profiling and the antibacterial activity of *Launaea procumbens* Roxb. Four different solvents viz., methanol, ethanol, chloroform and water were used for the extraction of plant leaves. The antibacterial activity was measured by the agar well diffusion method and determined by the Minimum Inhibition Concentration (MIC) against five pathogenic strains of bacteria viz., *Pseudomonas aureoginosa*, *Proteus mirabilis*, Klebsiella *pneumoneae*, *Acetobacter* and *Escherichia coli.* The potential of extracts, methanolic (9.46 mg/ml), ethanolic (6.17 mg/ml), chloroform (8.84 mg/ml) and water (14.34 mg/ml) was recorded by the agar well diffusion method and two well-known antibiotics Kenamycin (0.03 mg/ml) and Gentamycin (0.03 mg/ml) were used as the positive control. Among all the extracts, methanolic extract showed the maximum activity against almost all the species of bacteria, while the minimum activity was found to be of the aqueous extract. The phytochemical screening of the methanolic extract revealed the presence of alkaloids, phenols, tannins, flavonoids, steroids, glycosides and triterpenes in varying concentrations. Hence, the study could help out the use of plant leaves locally for the therapeutic purpose against bacterial infections as a potent antimicrobial source.

Keywords:- *Launaea procumbens* Roxb., Antibacterial analysis, Phytochemical analysis, Therapeutic purpose, Agar well diffusion method, Minimum Inhibition Concentration (MIC).

INTRODUCTION

The continuing emergence of drug resistance organisms and the increasing evolutionary adaptations by the pathogenic organisms to commonly used antimicrobial agents have reduced the efficacy of antimicrobial agents currently in use and therefore, the search for the new drugs from the novel sources such as plants continues to be necessary (Fransworth & Morris, 1976; Abdullahi & Lawal, 2010). Plants have been used since ages, for their therapeutic uses. In the earlier time it was not known regarding the chemical constituents of the plants but beliefs set to be used as the lifesaving remedy. Today the constituents of the different plants are been extracted and isolated for the same use in the name of medicines/drugs(Iwu, 2002). Several diseases that have been managed traditionally using medicinal plants include malaria, diarrhea, dysentery, bacterial and fungal infections, epilepsy, infantile convulsions and many other (Sofowara EA, 1982; Sofowara A, 1996).

These studies made the authors to study and analyze the plant *Launaea procumbens* Roxb., for phytochemical screening and antibacterial potential. The purpose behind the analysis for the antibacterial activity is because of the increasing incidences of multiple resistances in human pathogenic bacteria's in recent years due to the indiscriminate usage of commercially available antimicrobial drugs for the treatment of commonly affecting infections (Parekh & Chanda, 2007). Also although the synthetic and semi-synthetic antimicrobial

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drugs are absolutely present in the market, there is need for the continuous search for newer molecules to cope with the increased evolution of multiple antimicrobial resistance strains of organisms (Hart & Kariuki, 1998). The need of the hour is to find the best molecule among the all, by the specificity and the availability which can serve as the best broad spectrum antibiotic without any compromise with the efficiency.

The genus, *Launaea* belongs to the family Asteraceae and comprises about 40 species. Many of the species of the genus *Launaea* are used in the folk medicines in the treatment of skin diseases, tumors and dysentery (El-Bassuony & Abdel-Hamid, 2006). The studies on the genus with some species reveal the presence of specific phytochemicals which provides the plant, its medicinal importance (Sarg *et al*., 1982; Hook *et al*., 1984; Abd-El-Salam *et al*., 1986; Abd-El-Salam *et al*., 1990; Sokkar *et al*., 1993; Sarg *et al*., 1986; Saleh *et al*., 1998). Therefore in the present study, leaves of *Launaea procumbens* Roxb., were collected and extracted with the help of four different solvents viz., methanol, ethanol, chloroform and water, and analyzed for the antibacterial potential. The phytochemical screening was carried out to identify all the possible phytochemicals in the methanolic extract which had higher activity.

MATERIALS AND METHODS

Collection of Samples

Fresh plants was collected from, Shree Bapalal Vaidhya Botanical Graden, located in the Veer Narmad South Gujarat University Campus, Udhna Magdalla Road, Surat, Gujarat, India. Taxonomic identities of the plant were confirmed by the Taxonomists in Department of Biosciences, Veer Narmad South Gujarat University, Surat, Gujarat, India and the specimen voucher collection were preserved in the herbarium of the Department. The leaves from the plants were separated, washed under the running tap water and dried at 45°C in the oven. The dried leaves were then homogenized to fine powder and stored in the air tight container for future use. The bacterial strains were procured from the Microbial Type Culture Collection (MTCC) with the codes as follows; *Escherichia coli* (MTCC Code: 1683), *Pseudomonas aerugenosa* (MTCC Code: 4673), *Proteus mirabilis* (MTCC Code: 3310), *Klebsiella pneumoniae* (MTCC Code: 3384) and *Acetobacter spp*. (MTCC Code: 3245). All the strains of microorganisms were preserved at 4°C on nutrient agar slants.

Preparation of Extracts

Aqueous extract was prepared by adding 10gm of plant powder to the 100ml of distilled water. The solution was heated to boil using hot plate with intermediate shaking for about 25-30 min. The content was then filtered with Whatman No.1 filter paper and the filtrate was sterilized using autoclave and concentrated in a water bath at 60°C for about 2hr. A pinch of activated charcoal was added to remove the pigments. The concentrated decolorized extract was then stored at 4°C for future use (Adegoke & Adebayo-tayo, 2009).

Solvent extracts were prepared by adding 10gm of plant powder to each of the solvents viz., methanol, ethanol and chloroform in separate flasks. The solutions were then heated at 55°C on water bath for about 5min and then sealed with the glass stopper and kept on the rotary shaker for 24hrs. After 24hrs, the solutions were concentrated under reduced pressure at 45°C using the rotary evaporator to $1/10^{th}$ of the initial volume, and finally dried at 55°C in the oven. Dried extracts were weighted and stored at 4°C in refrigerator for future use (Parekh *et al*., 2005; Adegoke & Adebayo-tayo, 2009).

Antibacterial Analysis

Determination of antibacterial potential of the extracts was carried out using the agar well diffusion method (Perez *et al*., 1990). The molten nutrient agar media was poured in the sterile petriplates and inoculated evenly with the 0.1ml of the inoculum by sterile cotton swabs. 5 X 4 plates for each of the bacterial strains were prepared and four equal sections were marked on each plate. Four wells were prepared in each plate with the help of a sterile cork-borer of 8mm diameter. Each extract was diluted to obtain the concentration of 50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml using the sterile distilled water (Adegoke & Adebayo-tayo, 2009). 0.1ml of each diluted extract was then poured in the subsequent plates and incubated for 24hrs at 37°C. The subsequent plates of antibiotics viz., kenamycin and gentamycin for each bacterial strain were also prepared by the same method and incubated at 37°C for 24hrs. After the incubation period, zone of inhibition in each plate, for each concentration of extract and antibiotic (gentamycin and kenamycin) were measured by calculating the difference between diameter of cork-borer and diameter of inhibition (Hewitt & Vincent, 1989). The same method for experiment was carried out in the triplicates and the mean values were reported for the final consideration.

Determination of Minimum Inhibitory Concentration (MIC)

MIC of each extract was determined by the broth dilution method, where 0.1ml of standardized suspension of each bacterial strain, 114 CFU/ml, was added to each tube containing active fraction of 1-50mg/ml of the extract. The tubes were then incubated for 24hrs at 37°C. The lowest concentration of the tube that did not showed any visible growth by turbidity evaluation was considered as the MIC (Collins *et al*., 1995).

Phytochemical Screening

Based on the most effective extract in the antibacterial analysis, the qualitative screening for the phytochemicals was carried out using the methanolic extract. The tests for each phytochemical were carried out according to the, Table 2 (Harbone, 1973; Harbone, 1993; Trease & Evans, 1989).

RESULTS

Antimicrobial potential of the plant was determined by calculating the zone of inhibition of each of the extract. The results for the antibacterial studies are represented in the Table 4. It was found out that the methanolic extract was the most effective, as it showed antimicrobial activity against all the bacterial strains, while aqueous was the least [Table 4]. The table 5 represents the Minimum Inhibitory Concentration of the bacterial strains against all the prepared extracts. Phytochemical screening of the methanolic extract reveals the presence of phenols, tannins, flavonoids, alkaloids, steroids and cardiac glycosides in the plant at varying concentrations. The results of the phytochemical screening are given in the Table 3. The percentage yield of the organic solvents and water is given in the Table 1. The percentage yield in the Table 1 shows that the water is the most suitable solvent for the highest yield of the plant extract, but though the antimicrobial efficacy of the extract is least.

Table 1. Percentage yield of organic solvents and water

Sr. No.	Solvent	A in gm	B in gm	Percentage yield (%)
1.	Methanol	05	0.946	18.92
2.	Ethanol	05	0.617	12.34
3.	Chloroform	05	0.442	8.84
4.	Water	05	1.739	34.78
5.	Total yield			74.88

* A = Weight of powder plant material
B = Weight of extract
Percentage yield = (B/A) X 100.

Table 2. Procedural workout for the qualitative phytochemical screening

Sr. No.	Phytochemical	Test	Procedure	Inference
1.	Alkaloids	Dragendroff's test	0.5ml of extract + (2ml Dragendroff's reagent +1ml dil. HCL)	Orange precipitates
2.	Steroids	Salkowski test	0.5ml of extract + 1ml of Conc. H_2SO_4	Wine red color
3.	Cardiac Glycosides	Keller – Killani test	0.5ml of extract + Few drops of Glacial Acetic Acid (boil and cool) + 2 drops of $FeCl_3$ solution → Transfer the content slowly to the test tube containing 2 ml of Conc. H_2SO_4 from the wall of the test tube.	Reddish Brown ring at the junction of two solvents
4.	Phenols	Ellagic Acid test	0.5ml of extract + 2 ml of 5% (V/V) Glacial Acetic Acid/ 5% (V/V) $NaNO_2$ solution.	Muddy yellow/ Olive brown/ Niger brown/ Deep chocolate color
5.	Tannins	Tannin test	0.5ml of extract + 2ml of 15% (W/V) Gelatin.	White precipitation
6.	Lignans	Furfuraldehyde test	0.5ml of extract + 2ml of 2% (V/V) furfuraldehyde.	Red color
7.	Flavonoids	Flavonoid test	0.5ml of extract + Few Mg turnings + 2ml of Conc. H_2SO_4.	Magenta color (flavonoids) / Scarlet color (flavones) / Deep cherry color (flavonoids)
8.	Triterpenes	Libermann – Burchard's test	0.5ml of extract + Few drops of Acetic Anhydride + 1ml of Conc. H_2SO_4 from the side of test tube.	Red ring at the junction

Table 3. Results for the phytochemical screening

Sr. No.	Alkaloids	Steroids	Cardiac Glycosides	Phenols	Tannins	Lignans	Flavonoids	Triterpenes
1.	++	+	+	++	+	-	++	-

"++" = Present in appreciable quantity
"+" = Present in low quantity
"-" = Absent

Table 4. Antibacterial activity by agar well diffusion method of various extracts

Sr. No.	*Extracts*	*Concentration*	*Zone of Inhibition*				
			Escherichia Coli	*Psudomonas aerugenosa*	*Proteus mirabilis*	*Klebsiella pneumoneae*	*Acetobacter spp.*
1.	Methanolic	500mg/ml	23	22	25	29	25
		250mg/ml	18	18	20	19	18
		125mg/ml	14	14	16	15	15
		62.5mg/ml	11	11	13	11	9
2.	Ethanolic	500mg/ml	18	18	20	19	18
		250mg/ml	13	15	16	15	15
		125mg/ml	11	12	13	12	12
		62.5mg/ml	09	09	10	Na	09
3.	Chloroform	500mg/ml	15	13	11	10	12
		250mg/ml	13	11	09	Na	09
		125mg/ml	11	Na	Na	Na	Na
		62.5mg/ml	09	Na	Na	Na	Na
4.	Water (Aqueous)	500mg/ml	Na	Na	09	Na	09
		250mg/ml	Na	Na	Na	Na	Na
		125mg/ml	Na	Na	Na	Na	Na
		62.5mg/ml	Na	Na	Na	Na	Na
5.	Kenamycin	0.03mg/ml	35	33	43	48	28
6.	Gentamycin	0.03mg/ml	40	30	35	30	33
7.	Control	NA	Na	Na	Na	Na	Na

* All the figures are in millimeter (mm).
The zone of inhibition is including the diameter of cork borer i.e. 8mm.

Table 5. MIC of the extracts

Sr. No.	*Name of the Organism*	*MIC in mg/ml*			
		Methanolic Extract	*Ethanolic Extract*	*Chloroform Extract*	*Aqueous Extract*
1.	*Escherichia coli*	43	50	48	-
2.	*Psudomonas aerugenosa*	45	48	50	-
3.	*Proteus mirabilis*	41	45	50	-
4.	*Klebsiella pneumoneae*	38	43	50	-
5.	*Acetobacter spp.*	42	44	49	-

DISCUSSION

Extraction of the plant secondary metabolites depends upon the solvents used to extract them. It is well known that, in the traditional healing water is the primary source for the preparation of plant extracts, but for the instance we found methanol to be as the most appropriate solvent for the antimicrobial components of the plant. This might have resulted from the lack of solubility of active compounds in the water. Further, the phytochemical screening resulted in positive findings of the plant secondary metabolites which can serve to be as the bioactive compounds against the bacterial strains. The antimicrobial effect of the plant was lesser to the extent of the commonly employed commercial antibiotics, but this cannot be considered to be as the inefficacy of the plant. There can be some other compounds which may serve as antagonist for the effective bioactive compound (Jager *et al*., 1996). Further the studies could be directed to isolate the compounds and analyze for the potential of plant in the therapeutic purpose.

CONCLUSION

From the above procedural workout for the antimicrobial susceptibility testing of leaf extracts of *Launaea procumbens* Roxb., it can be concluded that the plant do have the potential for antimicrobial effect. Also it can be observed from the Table III, that methanol is the most suitable solvent for the extraction of plant material for antibacterial purpose. The phytochemical analysis of the methanolic extract confirms that the methanol, extracts the plant secondary metabolites which can be regarded as the source for antibacterial potential of the plant.

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