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"Phytochemical, Antibacterial and Antioxidant Activity of *Andrographis paniculata* Nees"

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Abstract: ***Andrographis paniculata Nees. commonly known as Kalmegh, used to treat infections and some deadly diseases like hepatitis, diabetes, cholera leprosy etc. In the present investigation, solvents like, Ethanol, Chloroform, Methanol and Distilled water were used to extract the phytochemical content and it revealed the presence of proteins, carbohydrates, phenols, tannins, glycosides, steroids and alkaloids. In vitro antibacterial activity against Gram-positive bacteria, Staphylococcus aureus and Bacillus cereus and Gram-negative bacteria, Escherichia coli and Klebsiella pneumoniae by agar well diffusion method. The results showed that the Methanol extract had significant inhibiting activity followed by Chloroform extract whereas the aqueous extract showed least zone of inhibition. The antioxidant assay was done for the plant extract and free radical scavenging potential was assessed by measuring its capability for scavenging nitric oxide radicals, hydrogen peroxide radicals as well as its ability in reducing power capacity assessment. Chloroform extract showed noticeable effect in the reducing power capacity. Methanol extract showed highest effect in hydroxyl radical and nitric oxide scavenging assay.***

Keywords: *Andrographis paniculata*, phytochemical analysis, antibacterial activity, agar well diffusion, antioxidant activity

1. Introduction

Herbal medicines are assumed to be of great importance in the primary healthcare of individuals and local communities in many developing countries [1]. Historians from all around the world have produced evidence to show that apparently all primitive people used herbs often in a sophisticated way [2]. Medicinal components from plants play an important role in conventional Western Medicine. The traditional medicine all over the world is nowadays revalued by an extensive study of results of research on different plant species and their therapeutic principles [3]. Interest in medicinal plants has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being and the bio-prospecting of new plant-derived drugs [4]. Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance [5].

Andrographis paniculata Nees. Commonly known as Kalmegh, Sanskrit: Kālamegha, is an herbaceous plant in the family Acanthaceae, native to India and Sri Lanka. The plant is used in blood purifying, leprosy, gonorrhea, scabies, boils, skin eruptions, and chronic and seasonal fevers. Juice or an infusion of fresh leaves is given to infants to relieve griping, irregular bowel habits, and loss of appetite. Pharmacological and clinical studies suggest the potential for beneficial effects in diseases like cancer and HIV infections [6]. In view of the above the present investigation was carried out to investigate Phytochemical analysis (qualitative) for the plant extracts obtained by Soxhlet extraction method, Antibacterial activity against Bacterial strains obtained from MTCC, Chandigarh and Antioxidant activity.

2. Materials and Methods

2.1 Sample collection and extraction

The plants were collected from Krupanidhi College and Gandhi Krishi Vignan Kendra, Bangalore. The plant materials were washed; shade dried and made into fine powder, 100grams of fine powder was weighed and extracted with solvents like Chloroform, Methanol, Distilled water using Soxhlet apparatus for about 30 to 40 cycles.

2.2 Preparation of test sample

The extracts were filtered, dried and 10mg of the dried sample was dissolved in 10ml of respective solvent (stock solution). 1ml of this solution was diluted to 10ml of respective solvent and the solution was further diluted to obtain 2-10µg/ml.

2.3 Phytochemical Screening

Qualitative phytochemical analysis of the chloroform, methanol and distilled water extracts of *A. paniculata* Nees were carried out using standard procedures to identify the constituent alkaloids (Mayer's test), steroids and terpenoids (Liberman- Burchard and Salkowski tests), cardiac glycosides (Keller-Kilani test), saponins (foam tests), flavonoids (Shinoda test), tannins and phenols (Ferric chloride test) as described by [7,8,9] Sofowara (1993), Trease and Evans (1989) and Harborne (1993).

2.4 Antibacterial Assay

In vitro antibacterial assay was examined for chloroform, methanol and distilledwater extracts of *A. paniculata* Nees. Using standard agar well diffusion method. 50mg of the samples were taken in 1ml of the respective solvent in petridishes. These plates were inoculated with 0.1 ml of two

Gram-positive bacteria: *Staphylococcus aureus* (MTCC3160) and *Bacillus cereus* (MTCC2155) and Gram-negative bacteria : *Escherichia coli* (MTCC443) and *Klebsiella pneumoniae* (MTCC3384) which were revived in nutrient broth media and incubated at 37°C for 24 hours. Each bacterial culture was maintained at 37°C on nutrient agar plates and nutrient broth after every 48 hours of sub-culturing.

2.5 Nitric Oxide Scavenging Activity [10]

Nitric oxide radical scavenging activity was done according to the procedure of Garret 1964 method and it was calculated according to the following formula:

$$\% \text{ Inhibition} = \frac{(AO - A1) \times 100}{AO}$$

AO =Absorbance of control, A1 = Absorbance of extract

2.6 Hydroxyl Radical Scavenging Activity [11]

The hydroxyl radical scavenging activity was performed using Yu *et al.,* 2004 procedure and the result was calculated by the following formula

$$\% \text{ Inhibition} = \frac{(AO - A1) \times 100}{AO}$$

AO =Absorbance of control, A1 =Absorbance of extract

2.7 Determination of antioxidant activity by reducing power assay

The reducing power assay test was performed according to the procedure [12] and the absorbance was measured at 700 nm.

3. Results & Discussion

3.1 Phytochemical Screening

The results of preliminary phytochemical analysis of plant extract revealed relative distribution of the secondary metabolite as shown in the Table 1 it also reveals us that there is highest amount of presence of alkaloids, flavonoids, carbohydrates, phenols, tannins and steroids this is also in confirmation with the leaves and stem extracts of *A. paniculata Nees*. [13]. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications [14, 15] revealed the inhibitory effect of saponins on inflamed cells. Steroidal compounds present in *A. paniculata* Nees extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones [16] .

Table 1: Screening tests for secondary metabolites in solvents extracts of *A. paniculata* Nees

Components	*Tests*	*Andrographis paniculata Nees.*		
		Chloroform	*Methanol*	*Distilled water*
Proteins	Millon's test	-	-	+
	Ninhydrin test	-	-	-
Carbohydrates	Fehling's test	+	+	+
	Benedict's test	-	-	-
	Iodine test	-	-	-
Phenols	Phenol test	-	+	+
Tanins	Tannin test	-	+	+
Flavonoids	Shinoda test	-	-	-
	Alkaline test	-	-	-
Saponin	Saponin test	-	+	-
Glycosides	Libermann's test	-	+	-
	Salkowski's test	-	-	+
	Keller-Kilani test	-	+	+
Steroids	Control	-	-	+
	Liberman-Burchard	+	-	-
Alkaloids	Mayer's test	-	+	-

3.2 Antibacterial activity observed in *Andrographis paniculata* Nees.

Antibacterial studies of the three different extracts (chloroform, methanol and distilledwater) of this plant revealed that the methanol extract (Fig 2) had significant activity against *Klebsiella pneumoniae* (50µl-12mm; 40µl-10mm and 30µl-4mm), *Bacillus cereus* (50µl-8mm; 40µl-6mm and 30µl-4mm) and *Staphylococcus aureus* (50µl-6mm; 40 &30µl-0mm). It had no activity against *Escherichia coli*. The extract using chloroform (Fig 1) showed activity only against *Staphylococcus aureus* (50µl-4mm; 40µl-2mm and 30µl-2mm) and did not show any activity against rest of the three bacterial species. It was observed that the distilledwater (Fig 3) extract had zero inhibition zones against all of the four bacterial species.

Figure 1: Zone of inhibition observed for chloroform extract against bacterial species

Figure 2: Zone of inhibition observed for methanol extract against bacterial species

Figure 3: Zone of inhibition observed for distilled water extract against bacterial species

The results are also similar to the earlier workers [17] worked on *A. bracteolate* found that the highest antibacterial activity in chloroform and methanol extracts this is also supported by the earlier workers [18].

3.3 Antioxidant Activity Analysis: 1. Nitric oxide scavenging activity

The nitric oxide scavenging activity of *A. paniculata* Nees. was found to be dose independent. The methanol extract showed highest nitric oxide scavenging activity followed by distilled water extract and chloroform in both the plants.

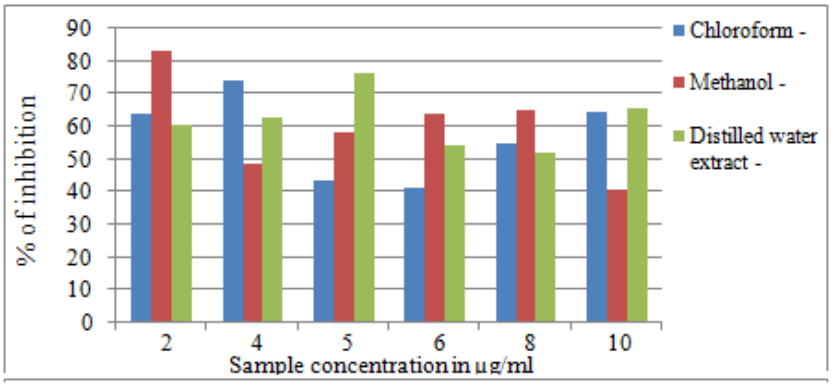

Figure 4: Graphical representation of % inhibition observed in nitric oxide scavenging activity

Fig 4 shows that in chloroform extract, the highest activity was found to be at 4µg/ml, in methanol extract at 2µg/ml and in distilled water extract at 5µg/ml. Also, the activity was found to be dose independent. Extracts of *A. paniculata* revealed the significant presence of antioxidative agents like flavonoids and tannins. Nitric Oxide (NO) scavenging assay is based on the scavenging ability of the extracts. The scavenging of NO was found to increase in dose dependent manner. Maximum inhibition of NO was observed in the extracts of highest concentration similar observations were found in *Ixora coccinea* [19].

3.4 Hydroxyl Radical Scavenging Activity

The results of hydroxyl radical scavenging activity exhibited activity in a dose independent manner. Percentage of inhibition was found in distilled water extract i.e. at 6µg/ml. Percentage of inhibition for *A. paniculata* was found to be highest at 2µg/ml level in methanol and distilled extract while no activity was found in chloroform extract (fig 5).

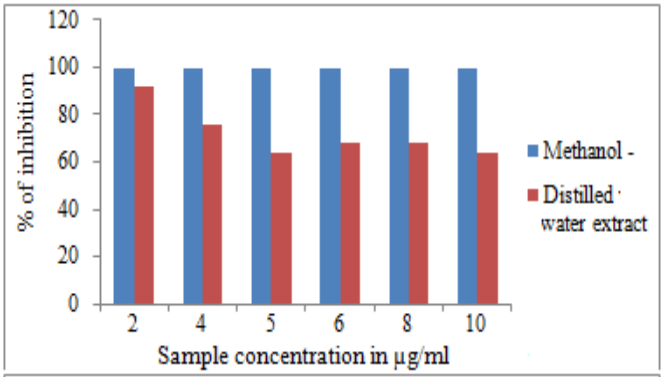

Figure 5: Graphical representation of % inhibition observed in hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity in *Abelmoschus spp* L [20] and found the activity using different solvents . Similar observations were also observed in methanol extracts of *kyllinga nemoralis* which showed scavenging activity on hydroxyl radicals ranged from 22.48 to 57.24% (50-250 µg) [21] .

3.5 Reducing Power Assay

The highest reducing power assay was shown by chloroform extract at 6µg/ml and the least by methanol extract at 4µg/ml (fig 6). Similar observations of reducing power assay and free radical scavenging effectiveness was observed in *Rosmarinus officinalis* L., and found higher reducing power in water soluble formulations [22] whereas in our study we found that, the more reducing power activity in methanol extract.

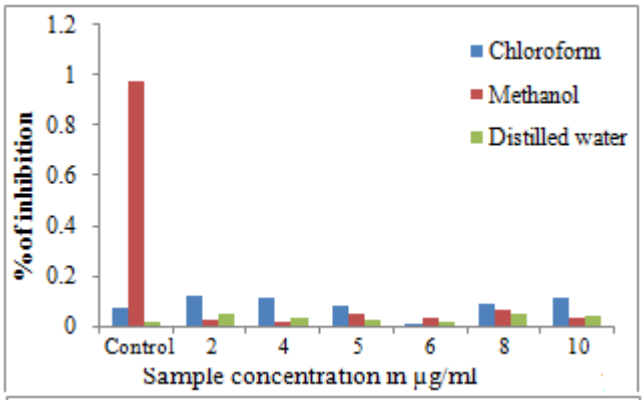

Figure 6: Graphical representation of % inhibition observed in reducing power assay

4. Conclusion and Scope for Future Work

The results show for the presence of phytochemicals which could be the responsible for the antioxidant property. The best results were obtained in Chloroform extract and showed noticeable effect in the reducing power capacity. Methanol extract showed highest effect in hydroxyl radical and nitric oxide scavenging assay. Further study is required to identity of the antibacterial compounds from the plant and also to determine their full spectrum of efficacy and it involves isolation and identification of chemical constituents.

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