Acknowledgement
ACKNOWLEDGEMENTS

Firstly, I wish to express my sincere and heartfelt thanks to my esteemed supervisor Dr. P.T. Kalaichelvan, Professor, CAS in Botany, University of Madras for his supervision, guidance, constant encouragement, and support during my study period. I thoroughly enjoyed the freedom given by him and I would like to acquire his tender.

I wish to extend my sincere gratitude to Prof. R. Rengasamy, Director, CAS in Botany, University of Madras for giving an opportunity to work in this centre and providing the laboratory facility.

I extend my sincere thanks to Dr. Devaki, Doctoral committee member, Department of Biotechnology, for his suggestion and criticism.

I also thank all other teaching and non teaching staff, CAS in Botany, University of Madras, for their help and support.

I am greatly indebted to Mr. M. Arulmani and G. Raja Mohan for his magnanimous support during my thesis preparation and throughout my study.

I am grateful to all my lab colleagues and my friends, in CAS in Botany for their kind help, encouragement and constant support during my entire research work. I express my sincere thanks to my all my school friends, college friends.
I gratefully acknowledge the UGC for the financial assistance provided under the scheme for meritorious students in science, which enables to pursue the research work.

The support, patience and encouragement unmindful of any inconvenience rendered by my family members, has enabled me to overcome the obstacles in the path of my progress. I have no words to express gratitude to my mother Mrs. A. Achikannu, my wife Mrs. V. Vijayarani, my brother Mr. A Rajakumar and his wife Mrs. R. Gayathri for their unmatched care and love. Without their co-operation and support, this could have never been possible.

A. VIJAYAKUMAR
ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY USING DIFFERENT EXTRACTS OF *ANACARDIUM OCCIDENTALE* L.

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**ABSTRACT:** The present study highlights the investigation of antioxidant and antimicrobial property of different extracts of seed coat and leaf of cashew nut (*Anacardium occidentale* L.). The antioxidant activity was determined by the 2, 2- diphenyl -1 picryl hydrazyl (DPPH) method. Maximum activity was observed in acetonic extract of *Anacardium occidentale* leaf which was 52.50% (1000µg/ml). The antimicrobial activity had been tested for the plant parts using its aqueous, acetone and ethanol extracts against two Gram-positive human pathogenic bacteria like *Micrococcus luteus* (lab culture), *Staphylococcus aureus* (MTCC96), four Gram-negative human pathogenic bacteria *Salmonella typhi* (ATCC12600), *Klebsiella pneumoniae* (MTCC109), *Escherichia coli* (MTCC1687), *Pseudomonas aeruginosa* (MTCC733). The ethanol extract of the seed coat of *Anacardium occidentale* L. were most efficacious against all the test organisms with zone of inhibition ranging from 12.0-34.0 mm, and the acetonic extract of the leaf sample of *Anacardium occidentale* L. was also active against all the test organisms with zone of inhibition ranging from 12.0-28.0 mm.

**Keywords:** *Anacardium occidentale* L, Anti-microbial & antioxidant activity, Human pathogens, Agar-well diffusion.

**INTRODUCTION**

The intent of the present contemplation matches the recent concept for ethnopharmacology, which is a “Study involving number of academic disciplines of the consistent physiological action of plant, animal and other matter used in innate medicines of past and present cultures” (International Society of Ethnopharmacology, 2005). Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centred free radicals and other reactive oxygen species that are continuously produced *in-vivo*, result in cell death and tissue damage. Oxidative damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis (Halliwell and Gutteridge, 1984). The degenerative diseases associated with aging include cancer, cardiovascular disease, immune-system decline, brain dysfunction and cataracts (Ames et al., 1993). As carcinogenic properties have been reported for some synthetic antioxidants, recent research on the potential applications of natural antioxidants from spices and herbs, for stabilizing foods against oxidation, have received much attention (Aruoma et al., 1996). The aggrandize in resistance of microorganisms due to multifarious use of commercial antimicrobial drugs encouraged scientists to scrutinize for new antimicrobial substances from various antecedents comprising medicinal plants (Karma et al., 2003). Various studies have looked at the antimicrobial activity of plant extracts. Considering the antimicrobial activity of *Anacardium occidentale* L. leaf and seed coat (skin of cashew nut). *Phyllanthus amarus* for its anti-hyperlipidemic activity (Van Holthoon, 2000) also indigenously for its hepatoprotective, anti-diabetic, antihypertensive, analgesic, anti-inflammatory and antimicrobial properties (Adeneye et al., 2006) analysis were determined on terpenes, alkaloids, lignans, flavonoids and tannins in *Phyllathus* species (Vongvanich et al. 2000). *Ocimum sanctum* L. (Labiateae) is commonly known as "holy basil", ‘Tulsi’, Cock et al 2008 reported the antimicrobial activity of *O. sanctum* leaves against bacteria and yeast. *Coleus forskohlii* is especially connoted for cardiovascular diseases appending hypertension; congestive heart failure and angina. *Mentha longifolia*, wild mint is a notorious acknowledged medicine, chiefly used for respiratory ailments.
In the traditional civilization, one of the plants which have been used for ethno medical purposes is *Anacardium occidentale* L. (cashew nut), belonging to Anacardiaceae family, and a native of Brazil, having great economic and medicinal value and which is composed of some 60 to 74 genera and 400 to 600 species. The leaves and seed coat of cashew tree has not been used as extensively as compared to many medicinal herbs or trees. It is not used as live stock feed for cattle also. Since the leaves and seed coat were not used effectively for any economical purposes, this study was undertaken to study its properties and ways to make it an economically important resource. *Anacardium occidentale* L. leaves, stem and bark extracts are utilized widely for the treatment of diarrhea, dysentery and colonic pain. (Bilcalho B, 2001). It has also been reported to possess anti-diabetic, anti-bacterial, anti-inflammatory and anti-ulcerogenic (Akinpelu DA, 2001). The leaves are also used in Brazil for eczema, psoriasis, scrofula, dyspepsia, genital problems, and venereal diseases, as well as for impotence, bronchitis, cough, intestinal colic, leishmaniasis, and syphilis-related skin disorders. The seed coat and the shell that remains after the extraction of the nut are used as fuel for burning purposes. This project is an attempt to explore the hidden potential of the seed coat and leaves to inhibit plant and animal pathogenic micro organisms and methods to enhance its activity, if found, to make it an economically useful product. The study reported that the bark extract revealed *in-vitro* antimicrobial activity against 13 of 15 microorganisms tested (O. O. Igbinosa et al., 2009). The plant produces many resources and products of importance. Antibiotic and other drugs when used in excess cause damaging effect to the health. But plant sources are comparatively less harmful to the human health. Hence plant resources are widely used for medicinal purposes. Here in this report the antifungal and antibiotic properties of cashew nut is been studied by taking plant fungal pathogens and human bacterial samples and studying the antagonistic activity of cashew seed coat and leaf extracts by agar diffusion method given by Kirby-Bauer (1966).

MATERIALS & METHODS

**Sample collection & Extraction**

*Anacardium occidentale* L. leaf and cashew skin coat were collected from cashew plantation areas of Elaikadambu r, Ariyalur district, Tamilnadu, India. The seed coat and the leaves were washed with running tap water and shade-dried for six days before they were powdered and kept in an airtight container prior to solvent extraction. 1 gram of the powdered seed coat sample and leaf samples were weighed and were soaked in 10 ml of water, ethanol (100%) and acetone and were kept over an orbital shaker at 150 rpm at room temperature for 48 hours. The acetone and ethanol extract were filtered with Whatman No. 1 filter paper, the residue discarded, and the filtrate was evaporated to semi solid state on a rotary evaporator at 40ºC. Since water is a non-volatile solvent, aqueous extract was lyophilized. The residue was suspended or dissolved in 1 ml of Phosphate Buffer Saline (pH 7.4). This was centrifuged at 5000 rpm for 5 minutes and the supernatant was collected and stored at 4ºC in airtight bottle until further use.

**Measurement of Antioxidant Activity**

The antioxidant activity of the crude methanol extract of the plant *Anacardium occidentale* belongs to Anacadiaceae, family, the antioxidant activity were determined on the basis of their free radical scavenging activity was measured *in vitro* by using of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH). A solution of DPPH (0.1 mM) in acetone was prepared, and DPPH was added to tested solution with solvents at different concentrations (100-700 µg/mL). After 30 min; the absorbance was measured at 517 nm using Beckman spectrophotometer. The percentage of free radical scavenging abilities at different concentrations was determined. D-Ascorbic acid was used as a standard. The DPPH absorbs at 517 nm, and its concentration is reduced by the existence of an antioxidant. The method described by Hatano *et al.* (1988); Bhuiyan *et al.* (2009) was used as per reference, from the difference in absorbance on DPPH; the percentage of inhibition was calculated as a function of antioxidant activity.
% radical scavenging activity = \frac{\text{absorbance of blank} - \text{absorbance of scavenging activity sample}}{\text{absorbance of blank}} \times 100

Test organisms & cultures
Cultures were obtained from the culture collection centre. (Centre for Advanced Studies in Botany, Guindy, Chennai, Tamilnadu) of which two were Gram-positive human pathogenic bacteria Micrococcus luteus, Staphylococcus aureus (MTCC96) and other four Gram-negative human pathogenic bacteria Salmonella typhi (ATCC12600), Klebsiella pneumoniae (MTCC109), Escherichia coli (MTCC1687), Pseudomonas aeruginosa (MTCC733) the organisms were brought MTCC and ATCC.

Anti microbial sensitivity test
The agar well diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Nutrient agar obtained from Himedia laboratories ltd (Mumbai). The Nutrient Agar plates were prepared by pouring 15-20 ml of molten media into sterile Petri plates; they were allowed to solidify for 5 minutes. Then 100 µl of the inoculum suspension was swabbed uniformly and it was allowed to dry for 5 minutes. Wells, with diameter of 7 mm, were cut on the surface of the plates (NA); different concentrations of extracts (50, 75 and 100 µl) were loaded into the wells. In NA the compound was allowed to diffuse for 60 minutes and the plates was kept for incubation at 37°C for 24 hrs. Then the % inhibition was calculated by the following equation:

RESULTS & DISCUSSION
The results confer the utility of Anacardium occidentale L. plant leaf extract in developing a novel broad spectrum antimicrobial agent.

Antioxidant activity
Anacardium occidentale leaf crude extract was assessed for their capacity to prevent the formation of 1, 1-diphenyl-2-picrylhydrazide (DPPH) peroxide radicals in a peroxgyenerating system as described. The antioxidant activity of the plant leaf extract shown in Table. 1. The standard D-Ascorbic acid which shows the activity of 61.71% at a minimum concentration of 6 µg/mL. When compare to standard Anacardium occidentale 52.50% (1000 µg/mL).

Antimicrobial activity
The 3 different concentrations (50, 75 and 100 µl) of the seed coat and leaf extracts showed varying degree of antimicrobial activities. M.luteus and S.aureus (MTCC96) showed 11 mm and E.coli (MTCC1687) showed 10 mm zone of inhibition with 100 µl of the Phyllanthus amarus aqueous leaf extract whereas the Anacardium occidentale L. aqueous leaf extract of 50 µl inhibited M.luteus with 13 mm, S.aureuse (MTCC96) with 10.5 mm, E.coli (MTCC1687) with 12.9 mm and P.aeuroginosae (MTCC733) with 11 mm zone of inhibition. Whereas the ethanol extract of P.amarus of 100 µl inhibited S.typhi (MTCC12600) with 15 mm and others with 10 -14 mm zone of inhibition comparatively 75 µl of the ethanol extract of A. occidentale seed coat and leaf inhibited E.coli (MTCC1687) with 13 mm and 33 mm respectively. 100 µl acetone extract of P.amarus inhibited M.luteus with 19 mm and the rest with 11-16 mm zone of inhibition. Whereas 100 µl of acetone extract of A.occidentale L. seed coat inhibited M.luteus and K.pneumonia (MTCC109) with 24 mm and the others with 19-21 mm zone of inhibition; and the leaf extract inhibited P.aureginosa (MTCC733) with 28 mm and the others with 19- 26 mm zone of inhibition.
Phytochemical analysis of *A. occidentale* L. Nuts: A variety of rich secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils are present in plants in general. The ethanolic extracts of *A. occidentale* L. shows the presence of various phytochemical compounds such as triterpenoids, phenolics and volatile oils. Ethyl acetate extracts exhibited a different combination of phytochemicals, phenolics, volatile oils, xanthoprotein and carbohydrates. Acetone found to be effective in dissolving the phytochemicals since many different compounds like triterpenoids, phenolics, volatile oils, flavonoids, xanthoprotein and carbohydrates were observed. However, Acetone, acted as good solvent for flavonoid extraction. The obtained results are in accordance with the reports of Tedong *et al.*, that phytochemical analysis of *A. occidentale* L. revealed the presence of alkaloids, polyphenols and saponins.

**Table 1: Antioxidant activity of Anacardium occidentale**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration (µg/mL)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>200</td>
<td>15.55</td>
</tr>
<tr>
<td>2.</td>
<td>400</td>
<td>22.22</td>
</tr>
<tr>
<td>3.</td>
<td>600</td>
<td>34.72</td>
</tr>
<tr>
<td>4.</td>
<td>800</td>
<td>41.66</td>
</tr>
<tr>
<td>5.</td>
<td>1000</td>
<td>52.50</td>
</tr>
</tbody>
</table>

The ethanolic extract contains high amount of terpenoids, phenols and volatile oils (Tedong *et al.*, 2006), and any of these compounds could be responsible for the inhibition of microorganisms.

The results from the present study showed that the two extract (acetone and ethanol) of *Anacardium occidentale* displayed efficacious antimicrobial activity against the considered 6 human pathogenic bacterial strains. As seen from the results (Table no. 2 & 3), both the extracts have showed a broad spectrum of activity. When the two crude extracts were compared with each other and with that of a standard antibiotic, Amikacin (30 mcg/ml), ethanolic extract of seed coat and acetonic extract of leaf samples was seen to possess a greater potential, compared to the other extracts.

**Table 2: List of pathogenic bacteria and diseases**

<table>
<thead>
<tr>
<th>Name of the pathogenic Organisms</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Micrococcus luteus</em> (Laboratory culture)</td>
<td>Septic shock, septic arthritis, endocarditis, meningitis, and cavitating pneumonia</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (ATCC C96)</td>
<td>Food poisoning and toxic shock syndrome and can cause bumble foot</td>
</tr>
<tr>
<td><em>Salmonella typhi</em> (ATCC12000)</td>
<td>Typhoid and paratyphoid fever</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> (ATCC109)</td>
<td>Notably pneumonia, septicemia ankylosing spondylitis.</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (ATCC1687)</td>
<td>Cholecystitis, Bactremia, Cholangitis, Diarthea</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> (ATCC733)</td>
<td>Urinary tract infection, ventilator associated pneumonia</td>
</tr>
</tbody>
</table>
Table 3: Vulnerability pattern of human pathogens to *Anacardium occidentale* seed coat and leaf extract (50 µl)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Aqueous extract (3 mg / 50 µl)</th>
<th>Acetone (3 mg / 50 µl)</th>
<th>Ethanol (3 mg / 50 µl)</th>
<th>Aqueous extract (3 mg / 50 µl)</th>
<th>Acetone (3 mg / 50 µl)</th>
<th>Ethanol (3 mg / 50 µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. luteus</em> (gram+)</td>
<td>13</td>
<td>20</td>
<td>24</td>
<td>13</td>
<td>21</td>
<td>18.5</td>
</tr>
<tr>
<td><em>S. aureus</em> (gram +)</td>
<td>10</td>
<td>16</td>
<td>21</td>
<td>10.5</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td><em>E. coli</em> (gram -)</td>
<td>12</td>
<td>17</td>
<td>26</td>
<td>10</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (g-)</td>
<td>-</td>
<td>18</td>
<td>12</td>
<td>-</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><em>S. typhi</em> (gram -)</td>
<td>-</td>
<td>14</td>
<td>23</td>
<td>-</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> (gram)</td>
<td>-</td>
<td>24</td>
<td>15</td>
<td>10</td>
<td>23</td>
<td>13</td>
</tr>
</tbody>
</table>

Acetone extract of the *Anacardium occidentale* L. leaf proved effective against all the test organisms with a maximum zone of inhibition of about 28 mm/100 µl against *Pseudomonas aeruginosa* (MTCC733). Zone of inhibition for *E.coli* was 17 mm/100 µl obtained for acetonic extract of anacardium leaf, was found to be greater compared to the chloroform + hydrochloric acid (13 mm/100 µl) extract of *Andrographis paniculata* (Soma roy et al., 2009) and higher than that given by methanolic extract of leaves of *Acacia nilotica* (15 mm), *Tinospora cordifolia* (14 mm) and *Sida cordifolia* (15 mm) (S.Satish et al., 2008). Whereas, ethanol extract of the seed coat proved effective against all the test organisms with a maximum zone of inhibition for *E.coli* (MTCC 1687) 34 mm/100 µl and for *Pseudomonas aeruginosa* (MTCC733) 31 mm/100 µl, which is even greater than the zone obtained from the leaf sample of *Anacardium occidentale* L.

This is one of the reports on the analysis of antioxidant and antimicrobial activity of crude compounds from different parts of *Anacardium occidentale* L. The antioxidant property was only checked with leaf extract whereas for the antimicrobial activities were tested against human pathogenic bacteria with both leaf and seed coat extracts. The results confer the utility of this plant extract in developing a novel d broad spectrum antimicrobial agent.

Table 4: Vulnerability pattern of human pathogens to *Anacardium occidentale* seed coat and leaf extract (75 µl)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Diameter of zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration of Seed coat extract (4.5 mg / 75 µl)</td>
</tr>
<tr>
<td></td>
<td>Aqueous extract</td>
</tr>
<tr>
<td><em>M. luteus</em> (gram+)</td>
<td>14</td>
</tr>
<tr>
<td><em>S. aureus</em> (gram +)</td>
<td>10</td>
</tr>
<tr>
<td><em>E. coli</em> (gram -)</td>
<td>13</td>
</tr>
<tr>
<td><em>K. pneumoniae</em> (g-)</td>
<td>-</td>
</tr>
<tr>
<td><em>S. typhi</em> (gram -)</td>
<td>-</td>
</tr>
<tr>
<td><em>P. aeruginosa</em> (gram)</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 5: Vulnerability pattern of human pathogens to *Anacardium occidentale* seed coat and leaf extract (100 µl)

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Concentration of Seed coat extract (6 mg / 100 µl)</th>
<th>Concentration of Seed coat extract(6 mg / 100 µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous extract</td>
<td>Acetone</td>
</tr>
<tr>
<td><em>M. luteus</em> (gram+)</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td><em>S. aureus</em> (gram +)</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td><em>E. coli</em> (gram -)</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td><em>K.pneumoniae</em> (g-)</td>
<td>-</td>
<td>24</td>
</tr>
<tr>
<td><em>S. typhi</em> (gram -)</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td><em>P.aeruginosa</em> (g -)</td>
<td>-</td>
<td>19</td>
</tr>
</tbody>
</table>

Graph 1. Antioxidant activity of *Anacardium occidentale* leaf

Graph 2. Vulnerability pattern of human pathogens to *Anacardium occidentale* seed coat (100 µl)
Conclusion

In this study supports the use of these plants in traditional medicine to treat various ailments like stomach complaints, wound infections and intestinal disorders etc. Anacardium occidentale L. exhibited apical antimicrobial activity in seed coat and leaf sample which is expected to be a renowned source of antimicrobial agents for the future endeavours.

REFERENCES


International Journal of Applied Biology and Pharmaceutical Technology Page: X
Available online at www.ijabpt.com


IN VITRO ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY SCREENING OF ANDROGRAPHIS PANICULATA LEAF ETHANOLIC EXTRACT IN TAMIL NADU

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Received: 22 Aug 2011, Revised and Accepted: 3 Oct 2011

ABSTRACT
This study is a positive demonstration on the utility of antimicrobial and antioxidant activities of leaf extracts of Andrographis paniculata Nees. From the leaf were extracted using various solvents such as Chloroform, Petroleum ether, Acetone, Ethyl alcohol, Isoamyl alcohol and Water (according to the non polar to high polar used for the extraction). The antibacterial activity was carried out against Micrococcus luteus, Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia by agar well diffusion method. The ethanolic extract was able to inhibit Escherichia coli, Staphylococcus aureus and Micrococcus luteus. The ethanolic extracts were screened for their in vitro antioxidant potential. Inhibition of oxygen derived free radicals, viz., assays for free radical scavenging by 2, 2-diphenyl-1-picryl hydrazyl (DPPH), reducing power ability and nitric oxide scavenging were performed. The antioxidant activity was compared with standard antioxidant such as D- ascorbic acid. The ethanol extract elucidated agreeable antioxidant and antimicrobial activity against four human pathogenic bacterial strains experimented.

Keywords: Ethnopharmacology, Andrographis paniculata, Antioxidant and Antibacterial microbial activity

INTRODUCTION
Medicinal plants are considerably useful and economically essential. They contain active constituents that are used in the treatment of many human diseases1. The plant extracts have been developed and proposed for use as antimicrobial substances2. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structures are different from those of the more studied microbial sources, and therefore their mode of action may too very likely differ3. There is growing interest in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity4, 5, 6, 7, and 8. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centred free radicals and other reactive oxygen species that are continuously produced in vivo, result in cell death and tissue damage. Oxidative damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis9. The degenerative diseases associated with aging include cancer, cardiovascular disease, immune-system decline, brain dysfunction and cataracts10. As carcinogenic properties have been reported for some synthetic antioxidants, recent research on the potential applications of natural antioxidants from spices and herbs, for stabilizing foods against oxidation, have received much attention11.

Andrographis paniculata also known commonly as "King of Bitters (English) or Hemped Bumi (Malay)," is a member of the plant family Acanthaceae. This is an herbaceous plant in the family Acanthaceae, native to India and Sri Lanka. It is widely cultivated in southern Asia, where it is used to treat infections and some diseases, often being used before antibiotics were created. Mostly the leaves and roots were used for medicinal purposes. This plant has been used for long without any known toxicity and has a strong traditional usage from safety point of view12. The herb has been used for the almost hundreds of years Asia for treating upper respiratory track infection, herpes and gastrointestinal track infection. It has also been used to treat hypertension, diabetic or as an anti-malarial12. The primary medicinal component of A. paniculata is andrographolide, which is a diterpene lactone. Andrographolide has been reported for its anti-cancer13, anti-HIV14, cardioprotective15 and hepatoprotective16 properties among others. The aim of the present investigation was to identify the compounds active for the antioxidant and antimicrobial activity of the leaf extract of A. paniculata.

MATERIALS AND METHODS

Andrographis paniculata Nees is an Herbal plant were collected from area of Elakkadambur village twenty one Kilo meter from Ariyalur District, Tamilnadu, India. The collected plants were identified and authenticated by Prof. P.T. Kalaichelvan, Life Science Department, University of Madras, Maraimalai Campus, Guindy and Chennai. The collected plant leaves were washed with running tap water and shade-dried for 3 days. After they were powdered and kept in an airtight container prior to solvents extraction. The extraction procedure is powder and each solvent in the ratio of 1:4. About the 100 g of dried leaves powder was extracted using 400ml of the extraction solvents with continuous shaking on a rotary shaker at 150 rev/min for 48 h and repeated three times. The filtrates were brought to slimy solid state by using hot water bath at 55 ºC and stored away at 4 ºC in air tight containers for further use. Slimy solid state paste dissolve with phosphate buffer saline then it was centrifuged at 5000 rpm for 5 minutes and the supernatant were subjected to further analysis.

Test organisms and cultures
Human pathogenic bacterial cultures were obtained from the culture collection centres (Centre for Advanced Studies in Botany lab, MTCC and ATCC). Two Gram-positive bacteria and two gram negative bacteria like Micrococcus luteus (CAS culture), Staphylococcus aureus (MTCC96), Escherichia coli (MTCC1687) and Klebsiella pneumoniae (MTCC109) respectively. All the microbial cultures were subcultured and maintained on Mueller Hinton Agar.

Determination of Antibacterial Activity
The agar well diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Mueller-Hinton agar medium was purchased from HiMedia, Laboratories Limited, Mumbai, India. The Agar plates were prepared by pouring 15-20 ml of molten media into sterile Petri plates; they were allowed to solidify for 5 minutes. Then 100 µl of the inoculum suspension was swabbed uniformly and it was allowed to dry for 5 minutes. Wells, with diameter of 7 mm, were cut on the surface of the plates; different solvent of extracts 100 µl were loaded into the wells. In the compound was allowed to diffuse for 60 minutes and the Mueller-Hinton agar medium plates were kept for incubation at 37ºC for 24 hrs.

Determination of Antioxidant Activity
The antioxidant activity was used this method described by Hatano\(^{18}\) and Bhuiyan\(^{19}\). Crude ethanolic extract of \textit{Andrographis paniculata} leaf was determined on the basis of their free radical scavenging activity was measured in vitro by using of the stable 1,1-diphenyl-2-picryl hydrazyl (DPPH). A solution of DPPH (0.1 mM) in ethanol was prepared, and DPPH was added to solvent dissolved test solution at different concentrations (200-1000 \(\mu\)g/mL). After 30 min, the absorbance was measured at 517 nm using Beckman spectrophotometer. The percentage of free radical scavenging abilities at different concentrations was determined. D-Ascorbic acid was used as a standard. The DPPH absorbs at 517 nm, and its concentration is reduced by the existence of an antioxidant. The difference in absorbance on DPPH and the percentage of inhibition was calculated as a function of antioxidant activity.

Then the % inhibition was calculated by the following equation

\[
\text{% radical scavenging activity} = \left(\frac{\text{absorbance of blank} - \text{absorbance of scavenging activity sample}}{\text{absorbance of blank}}\right) \times 100
\]

RESULTS AND DISCUSSION

The present attempt Isoamyl alcohol and Ethyl alcohol extracts showed the maximum antibacterial activity against all the four tested bacterial strains (Table 1 & Graph 1). Both gram positives and gram negative bacteria have much large diameter 3.9 cm in \textit{Micrococcus luteus} and 4.0 cm in \textit{Klebsiella pneumonia} than that of the tested bacteria. The Isoamyl alcohol extract having highest antibacterial activity was observed for the \textit{Klebsiella pneumonia} (4.0 cm) followed by \textit{Micrococcus luteus} (3.9 cm). Another one Ethyl alcohol was observed \textit{Escherichia coli} (2.9 cm), \textit{Staphylococcus aureus} (2.1) and \textit{Micrococcus luteus} (1.6). There was no inhibition was observed by water; acetone and petroleum ether extracts against \textit{Micrococcus luteus}, \textit{Klebsiella pneumonia} and \textit{Staphylococcus aureus}; \textit{Escherichia coli} having activity (petroleum ether 1.5 cm, chloroform 1.8 cm, acetone 1.6 cm, Ethyl alcohol 2.9, water 2 cm).

Isoamyl alcohol extract there is no inhibition against \textit{Escherichia coli}.

<table>
<thead>
<tr>
<th>Micro organisms</th>
<th>P.ether</th>
<th>Chloroform</th>
<th>Acetone</th>
<th>Ethanol</th>
<th>Water</th>
<th>L alcohol</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram positive bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{Micrococcus luteus}</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
<td>0</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>\textit{Staphylococcus aureus}</td>
<td>0</td>
<td>0</td>
<td>2.1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Gram negative bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{Escherichia coli}</td>
<td>1.5</td>
<td>1.8</td>
<td>2.9</td>
<td>2</td>
<td>0</td>
<td>4.0</td>
</tr>
<tr>
<td>\textit{Klebsiella pneumonia}</td>
<td>0</td>
<td>3.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

The above the results were measured in centimetre and triplicates

![Graph 1: Effect of Antimicrobial activity on different solvents](image)

Decolouration due to reaction of antioxidants in samples with the stable free DPPH radical was measured spectrophotometrically. Results (Table 2 & Graph 2) show the free radical scavenging potential of \textit{A. paniculata} leaf extract 91.01% (1000 \(\mu\)g/ml) is significantly lower DPPH free radical scavenging activity compared to the positive control 61.71% (6\(\mu\)g/ml).

<table>
<thead>
<tr>
<th>Concentration ((\mu)g/ml)</th>
<th>Percentage of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>28.74</td>
</tr>
</tbody>
</table>

Table 1: Effect of Antioxidant activity of \textit{Andrographis paniculata} L.
Among the different solvents Isoamyl alcohol were gave most large zone of the inhibitions compare to ethanol not large size of the zone but no of bacterium was inhibited. Other solvents moderate diameter of the results was observed. Zone of the results organisms’ vize, Micrococcus lettuce and Staphylococcus aureus bacteria ethyl alcohol extract only inhibited zone was observed. (1.6 cm, 2.1 cm) but all among them does not present the results 100 and 150µl concentrations.

Escherichia coli tested all the solvents present the results (1.5, 1.8, 1.6, 2.9 and 2 cm) and 1. alcohol not shown the result at 100 and 150µl concentrations.

Klebsiella pneumonia chloroform and Isoamyl alcohol solvent extract showed the results. (3.5 and 4.0 cm) but not inhibit petroleum ether, Acetone, ethan ol and water solvent extracts at 100 and 150µl concentrations.

Different µg/ml concentration of ethanolic extract (200µg to 1000µg/ml) was used for the estimation of antioxidant activity. Initially 200 to 400 µg/ml increases the working sample and inhibition also increased. Further 400 - 1000µg/ml concentration decreased ratio of the activity was showed. Finally 91.01% of maximum antioxidant activity was observed at the concentration of 1000µg/ml.

Present results showed interesting results it can concluded that the crude ethanolic extracts of Andrographis paniculata herbal plant leaves collected from Tamil Nadu unravelled are promising medicinal value like antibacterial and antioxidant activities. Further phytochemical work need to be done on these extracts including fractionation to isolate active constituent and subsequent pharmacological evaluation.

ACKNOWLEDGEMENT

Author thanks to Prof. R. Rengasamy, Director, CAS in Botany, University of Madras, for providing ample laboratory facility to carry out this research work and also thank UGC for providing (Science Meritorious Fellowship) financial support.

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