



Assessment of biological properties of two south Indian medicinal plants of *Bacopa monnieri* and *Anacardium occidentale*

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Abstract: The concern for the anti-microbial activity is increasing day by day due to the speedy growth of resistance towards various antibiotics by microbes, which is due to the improper consumption of drugs. India has got plenty of floras (medicinal plants) which helps widely in the anti-microbial and anti-oxidant research activities. The study of anti-microbial and anti-oxidant activity in the present case is done on *Bacopa monnieri* and *Anacardium occidentale*. Which is resulted in overcoming various bacterial strains due to high phenolic contents, as well as it consists of anti-oxidant properties which help in enhancing nerve impulse and also prevents the risk of cancer effects.

Key words: Anti-microbial activity; agar well diffusion; determination of total phenolic compounds; DPPH.

Introduction

The phytochemical follows a line of investigation based Ethnopharmacological information is generally considered an effective approach in the sighting of new anti-infective agents from higher plants. Historically plants have provided a good source of anti-infective agent. Medicinal plants are finding their way into pharmaceuticals, nutraceuticals, cosmetics and food supplements (Hussain J, *et al.*, 2009). Various pharmaceutical companies show interest in plant-derived drugs chiefly due to the current well-known belief that 'Green Medicine is safe and more reliable than the costly synthetic drugs, which have adverse side effects. As per the world health organization (WHO) report, 80% of the world population presently uses herbal medicine for some aspect of primary health care (Uniyal MR. *et al.*, 1968). The therapeutic value of plants lies in some chemical substances that produce a specific physiological action on the human body. In earlier days maximum peoples treated with medicinal plant extracts, especially in India. One of the most important plants Brahmi (*Bacopa monnieri*), and Cashew *Anacardium occidentale*. The most significant of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds (Yadav SK, *et al.*, 1989).

Bacopa monnieri:

Bacopa monnieri (BM) (also known as brahmi, water hyssop, *Bacopa monniera*, and *Herpestis monniera*), is a creeping perennial with small oblong leaves and purple flowers, found in warm wetlands, and native to Australia and India. Commonly found as a weed in rice fields, BM grows throughout East Asia and the United States (Barrett SC. *et al.*, 1978). The main nootropic constituents of BM are believed to be dammarane types of triterpenoid

saponins known as bacosides, with jujubogenin or pseudo-jujubogenin moieties as aglycone units (Sivaramakrishna C. *et al.*, 2005). Exhibit minimal observable adverse effects at standard dosages. BM demonstrates anti-oxidant (Tripathi YB. *et al.*, 1996), hepato protective, (Ghosh T. *et al.*, 2007), and neuroprotective activity (Rastogi M. *et al.*, 2012).

Recent studies have proved that its active constituent bacosides enhances the efficiency of nerve impulse transmission leading to improve memory related functions. It also has hepatoprotective, antidepressant, and antioxidant properties (Sumathy T, *et al.*, 2001; Kapoor LD: *et al.*, 1990). All the parts of the plant have been used for their therapeutic beneficiary effects from ancient times. It is used as memory enhancer (Morgan A, *et al.*, 2010), antidepressant, Adaptogenic agent (Chatterjee M, *et al.*, 2010), analgesic and anti-inflammatory (Channa S, *et al.*, 2006), anti-epileptic agent (Mathew J, *et al.*, 2011), nervine tonic (Pillai MM, *et al.*, 2009), anti-Parkinson's agent (Hosamani R, *et al.*, 2010), protective effect (Anbarasi K, *et al.*, 2006), cardiogenic agent (Ahmad R, *et al.*, 2010), anti-Alzheimer's drug (Uabundit N, *et al.*, 2010), anti-amnesic agent (Hsieh MT, *et al.*, 2010), anti-tumor agent (Peng L, *et al.*, 2010), anti-oxidant effects (Garg AN, *et al.*, 2009).

Oxidative stress (OS) occurs when free radicals (chemical species with unpaired electrons, produced during normal metabolism) overcome the cell's homeostatic defense mechanisms (Kregel CK. *et al.*, 2007). Protective, free radical-quenching enzymes include superoxide dismutase, catalase, glutathione peroxidase (GPx), glutathione reductase (GSR), and others. Anti-oxidant

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compounds also play a key protective role, including vitamins A, C, E, and myriad phytonutrients (particularly phenols) (Valko M. *et al.*, 2007; Rice-Evans C. *et al.*, 1997). Novel saponins called bacopasides I–XII have been identified more recently (Chakravarty AK. *et al.*, 2001; Garai S. *et al.*, 1996). The alkaloids brahmine, nicotine, and herpestine have been catalogued, along with d-mannitol, apigenin, hersaponin, monnierasides I–III, cucurbitacins and plantainoside B, (Chatterji N. *et al.*, 1965; Deepak M. *et al.*, 2005). The constituent most studied has been bacoside A, which was found to be a blend of bacoside A₃, bacopacide II, bacopasaponin C, and a jujubogenin isomer of bacopasaponin C. (Valko M. *et al.*, 2007).

Anacardium occidentale:

Cashew nuts, *Anacardium occidentale* L., belongs to the *Anacardiaceae* family and is an evergreen tree native from northeast region of Brazil which expanded spontaneously in South American countries (Asogwa *et al.*, 2008). African cashew nuts with shell are mostly processed in India and Vietnam (Ricard Rico *et al.*, 2015). One of the commonly consumed vegetables is the shoot of *Anacardium occidentale*. *A. occidentale* has been used to treat various ailments including malaria and yellow fever (Akinpelu, 2001) as well as diarrhea (Goncalves *et al.*, 2005). The biological activities of this plant is widely reported and it has been shown to possess anti-viral (Goncalves *et al.*, 2005), anti-fungal (Schmourlo, *et al.*, 2005), anti-bacterial (Akinpelu, 2001) and anti-inflammatory activities (Mota, *et al.*, 1985).

Cashew nut shell liquid (CNSL), a by-product obtained during the processing of cashew nuts is reported to possess antioxidant activity (Singh, *et al.*, 2004). The kernel of cashew nut valued in trade is covered with a thin reddish-brown skin or testa. The testa has been reported to be a good source of hydrolysable tannins (Pillai, *et al.*, 1963) with catechin and epicatechin as the major polyphenols (Mathew *et al.*, 1970).

As for its nutritional composition, phenolic lipids (Shobha *et al.*, 1992), saturated and unsaturated Fatty Acids, tocopherols, squalenes, and phytosterols (Ryan *et al.*, 2006), bioactive compounds such as β -carotene, lutein, zeaxanthin, α -tocopherol, γ -tocopherol, thiamine, stearic acid, oleic acid, and linoleic acid (Trox *et al.*, 2010) were already identified and determined in cashew nuts.

Anacardic acids (AAs) are abundantly present in many parts of the cashew plant and have received attention as a potential antioxidant substance. Cashew apple, cashew nut (raw and roasted), and cashew nut shell liquid (CNSL) contains a range of different alkyl phenols, including AAs, cardanols, and cardols. Higher amounts of AAs have been

detected in CNSL (353.6g/kg) followed by cashew fiber (6.1 g/kg), while the lowest (0.65 g/kg) amounts were found in roasted cashew nut (M. T. S. Trevisan, *et al.*, 2006). AAs were described as the main active agent in CNSL. The presence of a phytol side chain beside the phenolic ring structure (as in salicylic acid) results in its great antioxidant capacity. Diverse biological activities for the AAs have been described, including antimicrobial activity against methicillin-resistant bacteria (J. Kubo, *et al.*, 1999; H. Muroi *et al.*, 1996). It has been also demonstrated that AAs modulate the NF- κ B signaling pathway and inhibit tumor angiogenesis indicating that these compounds could be a therapeutic option in preventing or treating cancer (B. Sung, *et al.*, 2008; M. Hemshekhar, *et al.*, 2012). AAs from CNSL would prevent DEP-induced lung inflammation. Based on this hypothesis, they analyzed the potential anti-inflammatory and antioxidant properties of supplementation with AAs in a subacute model of DEP-induced inflammation in mice (Ana Laura Nicoletti Carvalho, *et al.*, 2013).

Materials and Methods

Plant Materials:

Collected medicinal plant *Bacopa monnieri* (whole plant), and *Anacardium occidentale* (leaves) was free from diseases collected from the regions of Visakhapatnam, Andhra Pradesh, India. The plant parts were cleaned of residual soil and air-dried at room temperature.

Chemical materials:

Methanol, Ethanol, Na₂CO₃ (Sodium bicarbonate), DMSO (dimethyl Sulphoxide), Distilled water, Nutrient agar, Potato dextrose agar, L- ascorbic acid.

Biological materials:

Bacterial strains:

Staphylococcus aureus, *Proteus vulgaris*, *Escherichia coli* and *Klebsiella pneumonia*.

Fungal strains:

Candida albicans, *Trichoderma mentagrophytes* and *Epidermophyton floccosum*.

Methanolic extraction by Soxhlet extraction method:

Dried (shade dried) *Bacopa monnieri* plant were grinded to powder form hence 100g of *Bacopa monnieri* sample were loaded to the cylinder (cylinder made with filter paper) of Soxhlet extractor, 600ml methanol were taken as a solvent. Temperature adjusted to 70°C gradually for prevention to solvent bumps. 46 cycles were observed each cycle average time period is 8.9 minutes. Methanolic extract allowed for dry into bacteriological incubator.

Preparation of plant extracts standard concentrations:

1 gm of each extract was taken and dissolved in 2 ml of DMSO (DiMethyl Sulphoxide). Thus 250, 100, and 50mg / ml of stock was obtained as a standard concentration of extracts.

Isolation of clinical pathogens:

Blood samples were collected from diseased persons in KGH (King George hospitals) Visakhapatnam. Patients with clinical positive cases and the samples were then transferred in culture bottles of brain heart infusion broth (Hi Media, Mumbai, India). Bottles were incubated at 36.7°C for 7 days. Bottles showing positive growth index were Gram stained and sub cultured on nutrient agar plates, all media were taken from Hi Media, Mumbai, India. These plates were aerobically incubated for 24-48 h at 37° in B.O.D.

Oral swab was taken by gently rubbing a sterile cotton swab over the infected body parts. The swabs were incubated in nutrient agar, and other selective media for primary isolation of the pathogens. These plates were then aerobically incubated for 24-48 h at 37°C. When growth was appeared on sub cultured plates of blood and oral swab, all bacterial pathogens were identified by standard microbiological and biochemical procedures. These biochemical tests include carbohydrates fermentation test, urease test, oxidase test, hemolysis of blood, catalase test, motility test and growth of pathogens on specific media etc. (Shimeld LA. 1998; Hawkey P, Law D, 2004; Ryan KJ. 2004; Mukherjee KL. 2006).

Screening of anti-microbial activity:

Anti-microbial activity using with agar well diffusion:

Autoclaved media poured into individual petriplates, before pouring media microbial strains inoculated to suitable media using with sterile inoculation loop (each bacterial strain inoculated to 20 ml of nutrient agar media, and each fungal strain inoculated to 20 ml of Potato dextrose agar medium then allowed petriplates for solidify after 10 minutes wells made with 6 mm

diameter cork borer and samples were loaded with help of micro pipette then allowed to incubation this microbial work has been done in bio safety chamber. after 24 hrs reports noted as zone of inhibition.

Estimation of total phenolic compounds:

100mg of methanol extracts were dissolved in 1ml of DMSO (Di Methyl Sulphoxide) and 20% Na₂CO₃sodium bi carbonate prepared for buffer solution. L-ascorbic acid was taken as standard and L- ascorbic acid prepared as six concentrations 500mg/ml, 400mg/ml, 300mg/ml, 200mg/ml, 100mg/ml and 50mg/ml. and allowed to incubation for 1hour in dark room after incubation reports noted under UV spectrophotometer absorbance at 780nm.

Determination for percentage of inhibition of anti-oxidant levels:

Percentage of scavenging free radicals of methanolic extracts determined by DPPH method. L-ascorbic acid taken as standard and distilled water & ethanol taken as control, and allowed to incubation for 20 minutes after incubation results noted absorbance at 520nm.

Results and Discussions

Anti-biotic treatment is based on determination of the aetiological agent and its relevant antibiotic sensitivity. Empiric treatment is often started before laboratory microbiological reports are available when treatment should not be delayed due to the significance of the disease. The effectiveness of individual antibiotics varies with the location of the infection, the ability of the antibiotic to reach the site of infection, and the ability of the bacteria to resist or inactivate the antibiotic. The significance of anti-bacterial and anti-fungal activity of *Bacopa monnieri* shown maximum zone of inhibition against *Klebsiella pneumonia* (24mm) and maximum zone of inhibition against on *Candida albicans*(19mm). *Bacopa monnieri* 20% shown maximum activity on *S. aureus* (16mm) and *C. albicans* (16mm).

Table 1: Anti-bacterial activity against bacterial strains

S. No.	Bacteria	<i>B. monnieri</i> 20%	<i>B. monnieri</i> Methanol extract			Standard (Ciprofloxacin)
			50mg/ml	100mg/ml	250mg/ml	
1	<i>E. coli</i>	11	7	9	12	28
2	<i>S. aureus</i>	16	8	9	12	19
3	<i>K. pneumonia</i>	13	8	19	24	29
4	<i>P. vulgaris</i>	12	11	12	15	34

Zone of inhibition of control 6 (in mm); 6mm Cork borer;

Table 2: Anti-microbial activity against fungal strains

S. No.	Fungi	<i>B. monnieri</i> 20%	<i>B. monnieri</i> Methanol extract			Standard (Flucanazole)
			50mg/ml	100mg/ml	250mg/ml	
1	<i>C. albicans</i>	16	14	16	19	11
2	<i>T. mentagrophytes</i>	11	8	9	11	23
3	<i>E. floccosum</i>	12	8	9	10	9

Zone of inhibition of control 6 (in mm); 6mm Cork borer;

Table 3: Anti-bacterial activity of *Anacardium occidentale*:

S. No.	Bacteria	<i>Anacardium occidentale</i> Methanol extract			Standard (Ciprofloxacin)
		50mg/ml	100mg/ml	250mg/ml	
1	<i>E. coli</i>	7	8	16	26
2	<i>S. aureus</i>	7	9	10	22
3	<i>K. pneumonia</i>	6	7	12	29
4	<i>P. vulgaris</i>	7	8	18	34

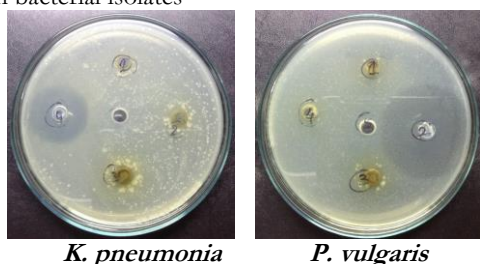
Zone of inhibition of control 6 (in mm); 6mm Cork borer;

Table 4: Antifungal activity of *Anacardium occidentale*:

S. No.	Fungi	<i>Anacardium occidentale</i> Methanol extract			Standard (Fluconazole)
		50mg/ml	100mg/ml	250mg/ml	
1	<i>C. albicans</i>	6	6	11	22
2	<i>T. mentagrophytes</i>	9	10	12	21
3	<i>E. floccosum</i>	8	9	10	09

Zone of inhibition of control 6 (in mm); 6mm Cork borer;

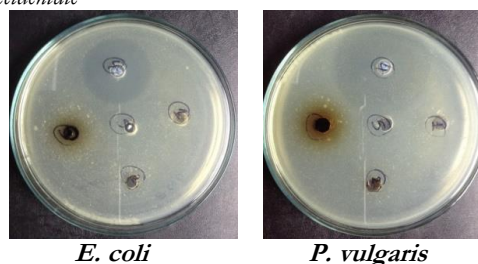
Figure 1: Anti-bacterial activity of *Bacopa monnieri* on bacterial isolates



K. pneumonia

P. vulgaris

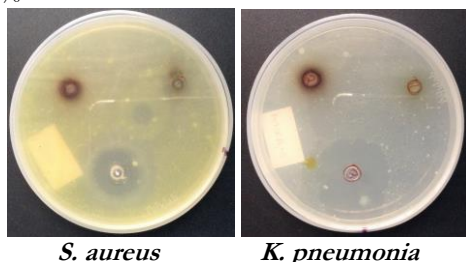
Figure 5: Anti-bacterial activity of *Anacardium occidentale*



E. coli

P. vulgaris

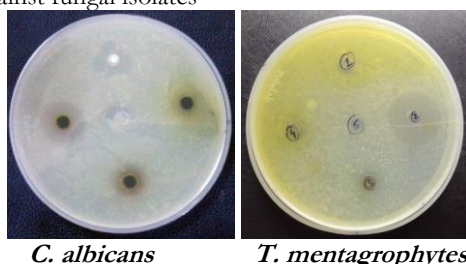
Figure 2: Anti-bacterial activity of *Bacopa monnieri* 20%



S. aureus

K. pneumonia

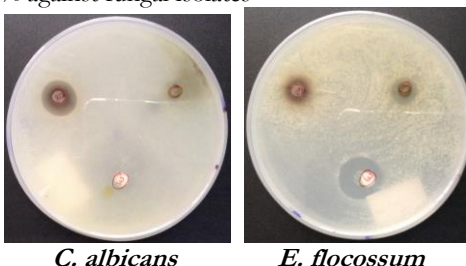
Figure 3: Anti-fungal activity of *Bacopa monnieri* against fungal isolates



C. albicans

T. mentagrophytes

Figure 4: Anti-fungal activity of *Bacopa monnieri* 20% against fungal isolates



C. albicans

E. floccosum

Evaluation of total phenolic compounds in methanolic plant extracts:

Table 5: Determination of total phenolic compounds

Concentration In Mg/MI	Total Phenolic Compounds In µg/MI
<i>Bacopa monnieri</i> 1mg/ml	346
<i>Bacopa monnieri</i> 20% 1mg/ml	302
<i>Anacardium occidentale</i> 1mg/ml	235

Determination of anti-oxidant levels in methanolic plant extracts

Table 6: Percentage of inhibition of plant extracts by DPPH method.

Concentration in µg/ml	Percentage of inhibition
<i>Bacopa monnieri</i> 250 µg/ml	34%
<i>Bacopa monnieri</i> 20% 250 µg/ml	37%
<i>Anacardium occidentale</i> 250 µg/ml	43%

The Concentration of 250mg/ml of *Bacopa monnieri*, *Bacopa monnieri* 20%, and *Anacardium occidentale* exhibited considerable growth zone while the total phenolic content in *Bacopa monnieri* was found 346µg/ml, *Bacopa monnieri* 20% was found 302µg/ml, and *Anacardium occidentale* was found 235µg/ml. And the percentage of inhibition was determined by DPPH method to be *Bacopa monnieri* 34%, *Bacopa monnieri* (20%) have 37%, and *Anacardium occidentale* to be 43%. These results showed the antibacterial and antifungal activity of various bacterial and fungal strains and helping to study the multiple drug resistivities of the isolated bacterial and fungal strains.

Discussions

Antimicrobial activity from plant source can be assumed to be useful. The extract produces anti-

infective agent which could be active against human pathogens (Gupta MP, *et al.*, 2005). Apart from antimicrobial activity exhibited by tannins, they also lead with proteins to provide the typical turning effect. Medicinally, this is important for the treatment of inflamed tissues (Mota MLR, *et al.*, 1985).

The ethanol extracts of *Anacardium occidentale* have inhibited the growth of Gram negative and Gram positive bacteria. Moreover, the *Anacardium occidentale* leaves aqueous extract were known to make, at 200 mg/ml an inhibitory effect on growth of *S. aureus*, and *E. coli* (Omojasola and awe 2004)

Conclusion

In this study the methanolic extracts of *Bacopa monnieri* and *Anacardium occidentale* results were shown against microbial activity, because presence of phenol contents, those are more potential to anti-microbial properties can easily accessible source of natural anti-microbial and as a possible food supplements, and pharmaceutical industries. *Bacopa monnieri* is a broad spectrum agent which can be used against gram positive and gram negative bacteria and fungal organisms (*Candida albicans*, *Epidermophyton floccosum*, and *Trichoderma mentagrophytes*). And both *Bacopa monnieri* and *Anacardium occidentale* have radical scavenging activity which is called anti-oxidants.

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