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ETHNO-MEDICINAL EXPLORATION OF *BUXUS PAPILLOSA* C.K. SCHNEID. (ANTIOXIDANT, ANTIMICROBIAL AND ANTHELMINTIC ACTIVITIES)

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ABSTRACT

In the present study, the antimicrobial, antioxidant and anthelmintic activities of the plant *Buxus papillosa* C.K. Schneid. were evaluated. The crude extracts of powdered plant material were taken in a series of polar and nonpolar solvents, petroleum ether, chloroform, methanol and distilled water. The plant was found potent against pathogenic microbes, i.e. *Staphylococcus aureus, Staphyllococcus saprophyticus, Escherchia coli* and *Pseudomonas aeruginosa*, *Aspergillus parasiticus* and *Rhizopus oryzae,* because well-defined zones of inhibition were recorded. The antioxidant activity of all the plant extracts was studied by DPPH Assay, total antioxidant assay and total phenolic assay and the remarkable values comparable with the standard antioxidants (BHT and α Tocopherol) were recorded. For the determination of anthelmintic activity standard Levamisole was run against *Haemonchus contortus* worms and compared with all the extracts.

Key Words: Antimicrobial; Minimum Inhibitory Concentration; Phenolic; Standards

INTRODUCTION

Often the herbs and medicinal plants are used as the first medicines. Every culture on the earth either written or oral tradition, all has relied on the immense variety of natural chemistrie's found in plants for their therapeutic properties. All drugs that are extracted from the plant are substances with a particular therapeutic action. About 80% of the world population in Asia, Latin America and Africa used herbal plants as traditional health remedies and reported minimal side effects (Doughari, 2006). *Buxus papillosa* C.K.Schneid. (Syn..*Buxus semipervirens* Sensu Stewart and Brandis). It is generally known as Boxwood and belongs to family Buxaceae. It is an ornamental small trees or a shrub grown chiefly for its handsome evergreen foliage (Nasir and Ali, 1974). Its flowering period is from January-May and is very rich in phytochemicals as Buxapapillosin, Buxamine A, Buxinidine (Shinwari and Rehman, 2006).

Tshikalange *et al*. (2008) investigated the Ethanol extracts of eight plant species that were used traditionally in South Africa for the treatment of oral diseases for *in vitro* antimicrobial activity against oral pathogens namely *Actinobacillus actinomycetemcomitans, Actinomyces naeslundii, Actinomyces israelii, Candida albicans, Porphyromonus gingivalis, Privotella intermedia* and *Streptococcus mutans* using the disk diffusion method. Using micro dilution, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ethanol extracts were determined against these microorganisms. The cytotoxicity and therapeutic index (TI) of particular active extracts were also determined. Out of eight plants, six (*Annona senegalensis, Englerophytum magalismontanum, Dicerocarym senecioides, Euclea divinorum, Euclea natalensis, Solanum panduriforme* and *Parinari curatellifolia*) showed MIC values ranging from 25.0 mg/mL to 0.8 mg/mL. Except for *Euclea natalensis* which inhibited all three Gram negative bacteria tested in this study while Gram negative bacteria were found to be more resistant to the plant extracts than Gram positive bacteria,. All plant extracts showed moderate cytotoxicity on the Vero cell line. The 50 % inhibitory concentration (IC_{50}) of all plants tested range from 92.3 to 285.1µg/mL.

Villagomez *et al*. (2005) specially emphasized on the seed cones and evaluated the total phenolic content and antioxidant activity of extracts from pine species for various plant components. Seed cones from pine species were found to contain comparatively high amounts of both total phenolics and antioxidant activity, appears to be some correspondence of the two measurements. Juvenile cones contained by far the maximum phenolic and antioxidant activity, but this high activity appears to be related to seeds reserved in some of the samples, probably due to proteins or additional antioxidants that were present in the seeds. The phenolic content and antioxidant activity were also measured for other plant components and for a few other species for comparison. In general, the cones of red

and jack pine showed the maximum antioxidant activity as compared to black and southern pines. The general trend by phenolics was: juvenile cones > needles > new cones > bark > old cones > wood.

Obviously, cones could represent a viable source of antioxidants, especially compared to the wood of species that had moderately low activity. Collection and extraction of pine cones for antioxidants would be a non-destructive method for procurement of this medicinal component.

MATERIALS AND METHODS

The plan of work was designed to estimate the pharmacological effects of all the plant specimens under following steps:

- Collection and preservation of the plant material
- Solvent Extraction by Maceration Method in the Polar and Non polar solvents e.g. Petroleum ether, Chloroform, Methanol and Double Distilled Water.

The plant material was collected from the GCU Botanic garden during the month of February. All the plant materials were authenticated by Prof. Dr. Zaheer-ud-Din Khan, Chairman Department of Botany. The Voucher specimens of all the plant specimens were submitted to Dr. Sultan Ahmed Herbarium, Department of Botany, GC University, Lahore.

Antimicrobial activity

Antimicrobial activity of the plant extracts according to Ortega *et al*. (1996) and Ferreira *et al*. (1996). Media preparation was done according to Johnsen (1940) and Qadeer *et al.* (1999). Minimum Inhibitory Concentrations (MIC) were calculated following Murray *et al.* (1996).

For comparison of antimicrobial activity certain commercially available antimicrobial discs were used as: Ampicillin disc (10 µg) against *Staphylococcus aureus*, Ampicillin disc (10 µg) against *Staphylococcus saprophyticus*, Amikacin disc (30 µg) against *Pseudomonas*, Sulphomethoxazole disc (23.75 µg) against *E.coli*, Fucanozole medicine in the form of dilution as 250mg/625mL against *Aspergillus parasiticus* and *Rhizopus oryzae*

Antioxidant activity

For antioxidant evaluation of the plant extracts, DPPH assay and Total antioxidant assay were applied. The details of the methods used for antioxidant activity were as follows: DPPH assay was done according to according to Erasto *et al*. (2004) and percentage scavenging activity was determined by applying following formula

SC% = [1-(absorbance of samle) / (absorbance of control) ×100.

According to the method of Prieto *et al*. (1999), the total Antioxidant capacity of all the extracts was analyzed. The total Phenolics of all the plant specimens were determined by the method of Makkar *et al*. (1993). For comparison the standards BHT and α Tocopherol were run.

Anthelmintic activity

This activity is mainly concerned with the use of plant specimens in the Ethnoveterinary system of medical sciences in Pakistan.

In this aspect the plant extracts (0.5mg/mL) were studied *in vitro* was and for this purpose live *Haemoonchus contortus*, intestinal parasite of sheep were used by following the methodology of Sharma *et al*. (1971), Lal *et al*. (1976) and Singh *et al.* (1985) with a little modifications in the methodology of Fasiuddin and Campbell (2000).

The comparison was made with the standard anthelmintic medicine Levamisole (0.55mg/mL). Whole experimental set up was run in Randomized Complete Block Design (RCBD).

RESULTS

The inflorescence water extract of *Buxus papillosa* produced the maximum zone of inhibition (46 ± 0.577) against *S. aureus*, while the fruit chloroform extract showed the least value of zone of inhibition (6 ± 0.269) among all the extracts against all the microbial strains. The leaf water extract of *B. papillosa* produced the highest inhibition value (68 ± 1.26) against *Rhizopus oryzae* whereas the lowest value was observed in case of inflorescence chloroform extract against *A. parasiticus* (21 ± 0.5). A comparison was made among all the plant specimens for their antimicrobial activity, and the *Buxus papillosa* leaf distilled water extract showed very high inhibition with the value (68 ± 1.26) against *R. oryzae*. In the same way the standard antimicrobial discs were also used for comparison with the plant extracts and they all showed their characteristic zones of inhibition. Ampicillin showed the zones of inhibition against the *S. aureus* and *S. saprophyticus* having the values 23.6 ± 3.51 and 33.6 ± 3.51 respectively.

Sulphomethoxazole produced zone of inhibition (25.91 ± 2.12) against *E. coli.* Amikacin produced its inhibitory zone against *P. aeruginosa* with the value 13.66 ± 3.21. Fuconazole was used against *A. parasiticus* and it inhibited the fungal growth, producing the area of 32.33 ± 2.12 mm. Kanamycin formed (53.66 ± 1.20) zone of inhibition against *R. oryzae*.

Minimum Inhibitory Concentration (MIC)

MIC value for *Buxus papillosa* methanol leaf extract was measured as 0.7µL.

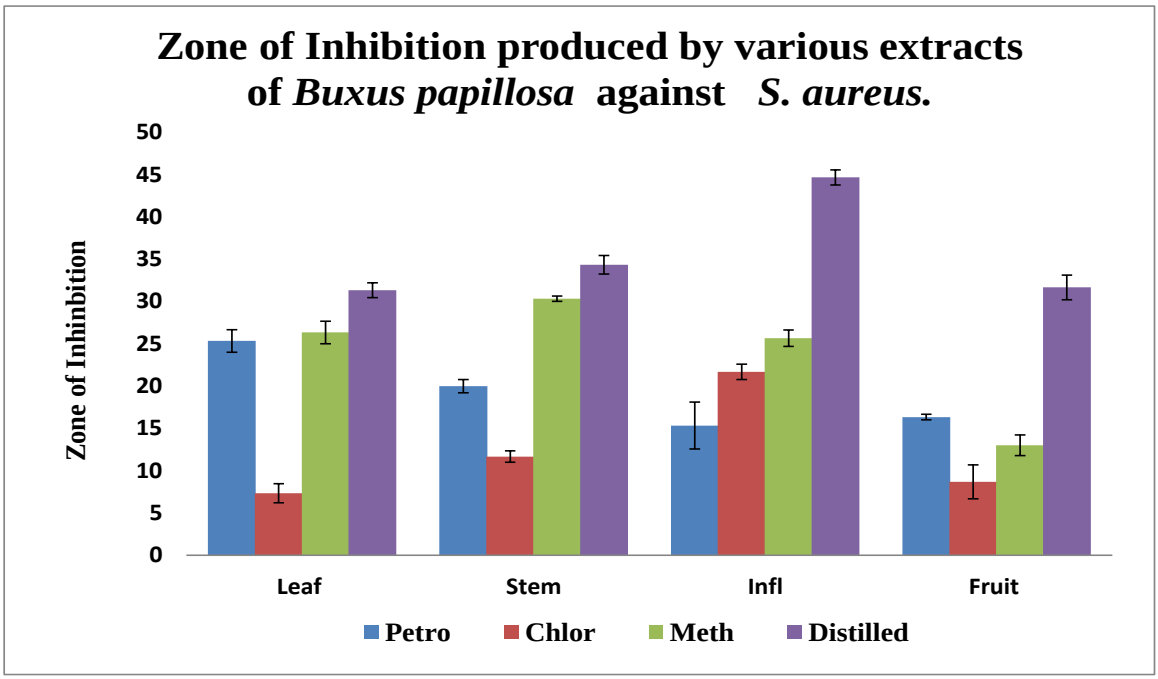

Fig 1. Zone of inhibition (mm) produced by extract of *B. Papillosa* against *S. aureus.*

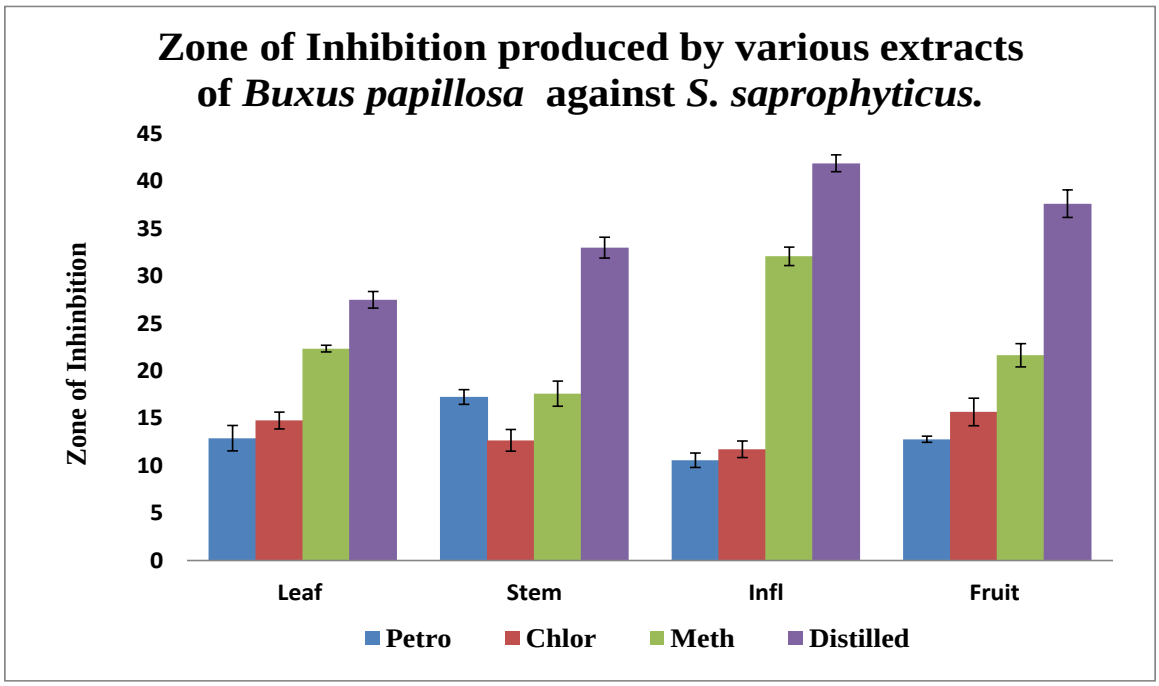

Fig. 2. Zone of inhibition (mm) produced by extracts of *B. Papillosa* against *S. saprophyticus*

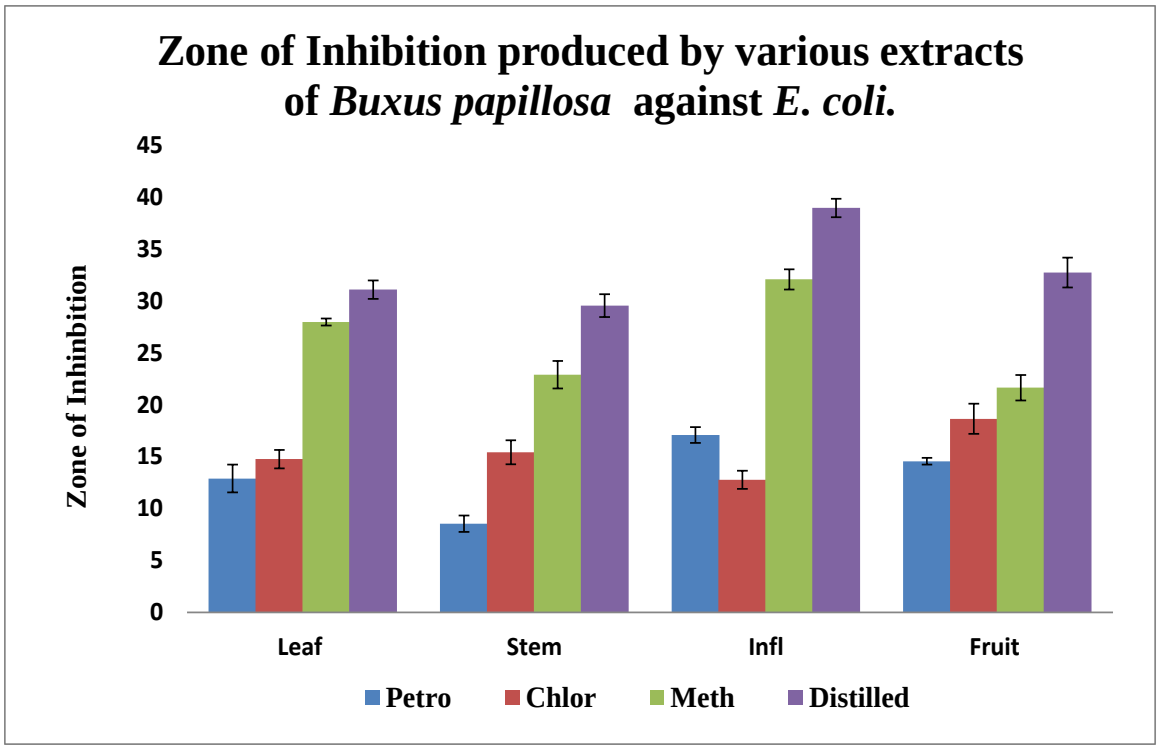

Fig. 3. Zone of inhibition (mm) produced by extracts of *B. Papillosa* against *E. coli.*

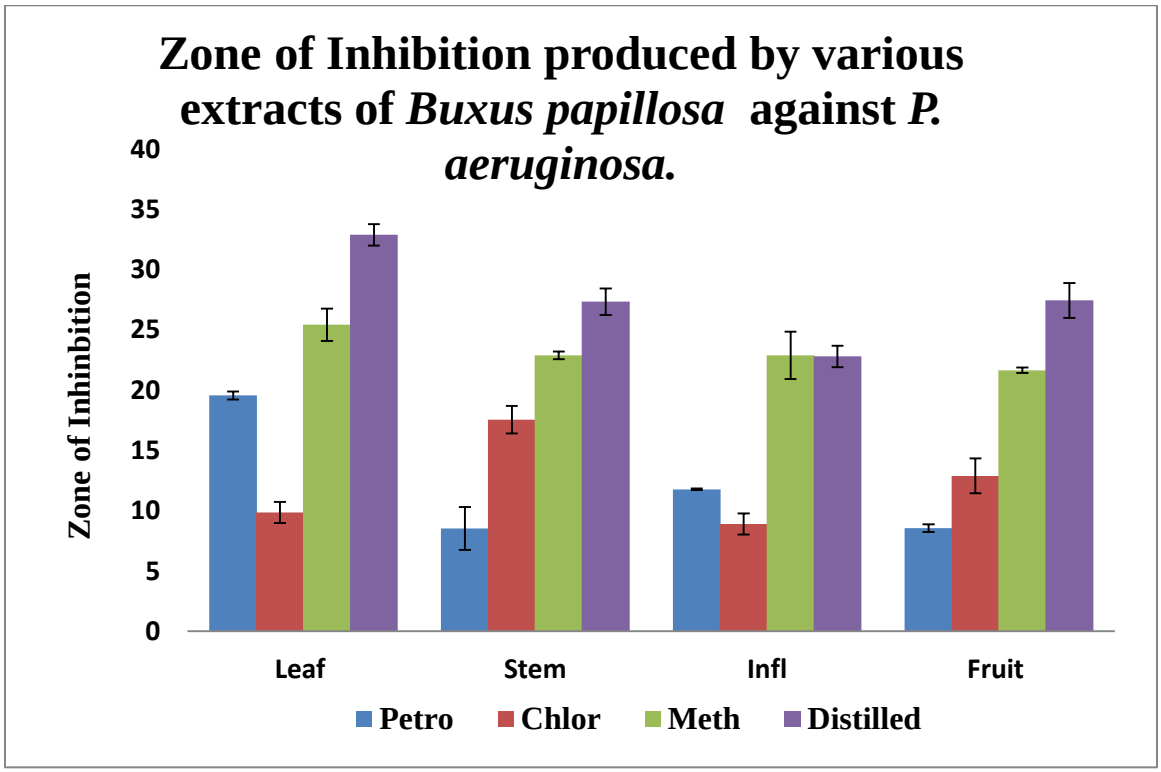

Fig. 4. Zone of inhibition (mm) produced by extracts of *B. Papillosa* against *P. aeruginosa*

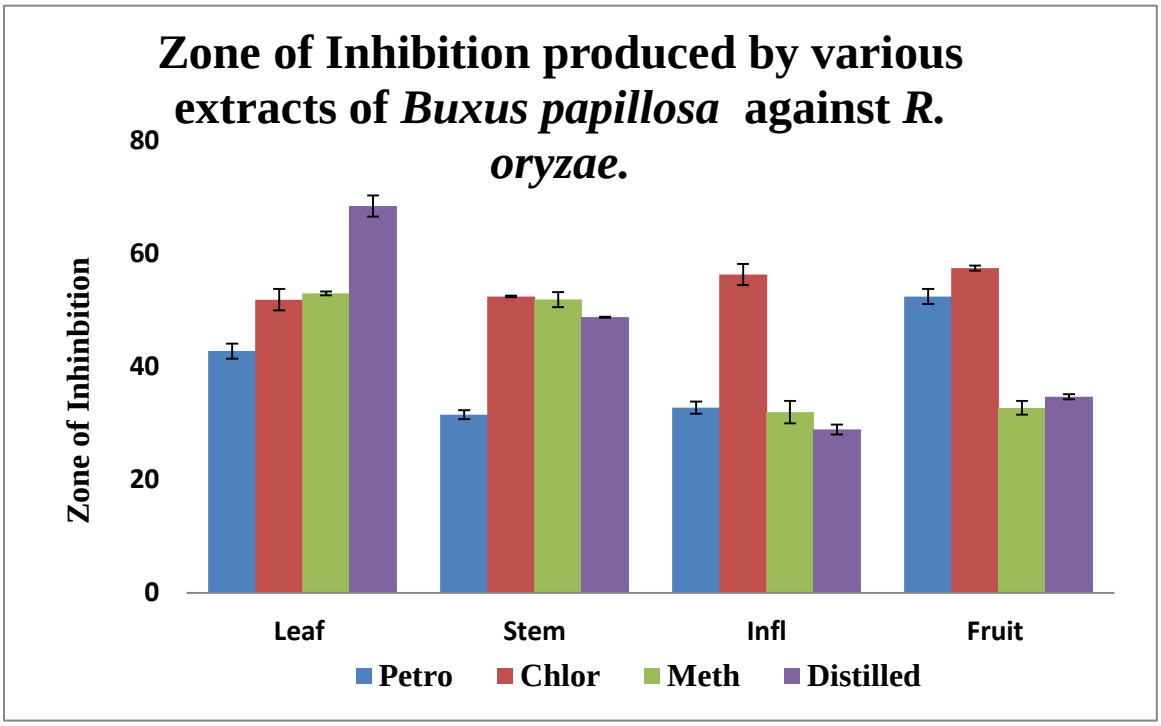

Fig. 5. Zone of inhibition (mm) produced by extracts of *B. papillosa* against *R. oryzae*

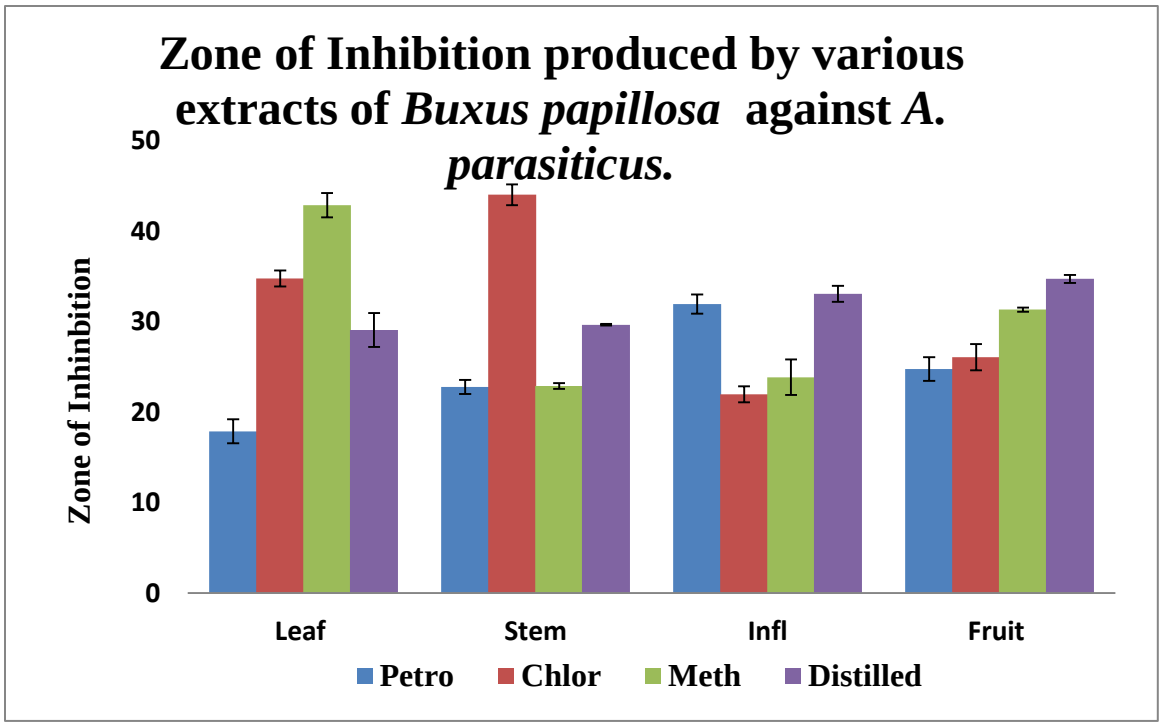

Fig.6. Zone of inhibition (mm) produced by extracts *B. Papillosa* against *A. parasiticus.*

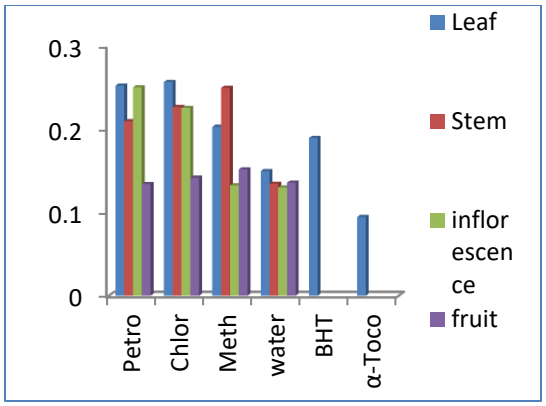

Fig. 7(a)

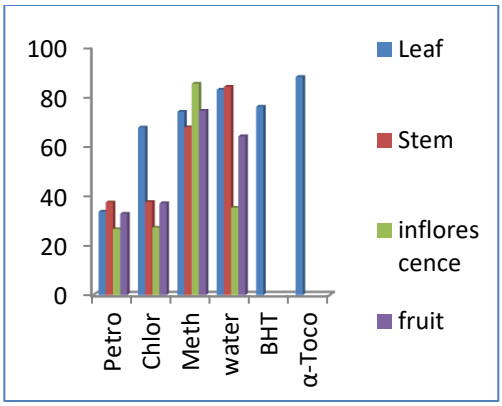

Fig. 7(b)

Fig. 7(a). Antioxidant activity through DPPH Assay (b) Percentage DPPH values of various extracts of *B. papillosa* in different solvents.

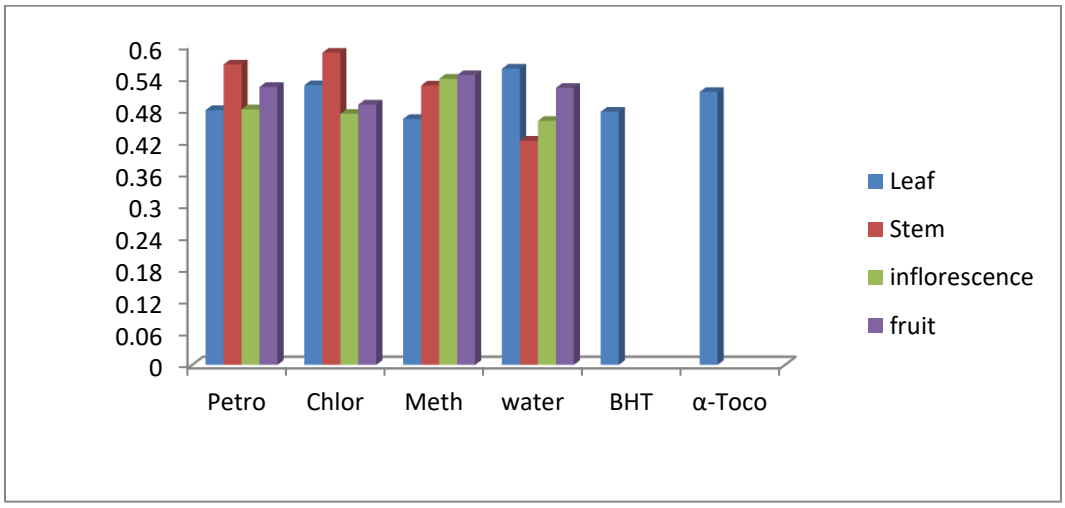

Fig. 8**.** Antioxidant activity of various extracts of *B. papillosa* in different Solvents through Total Antioxidant Assay.

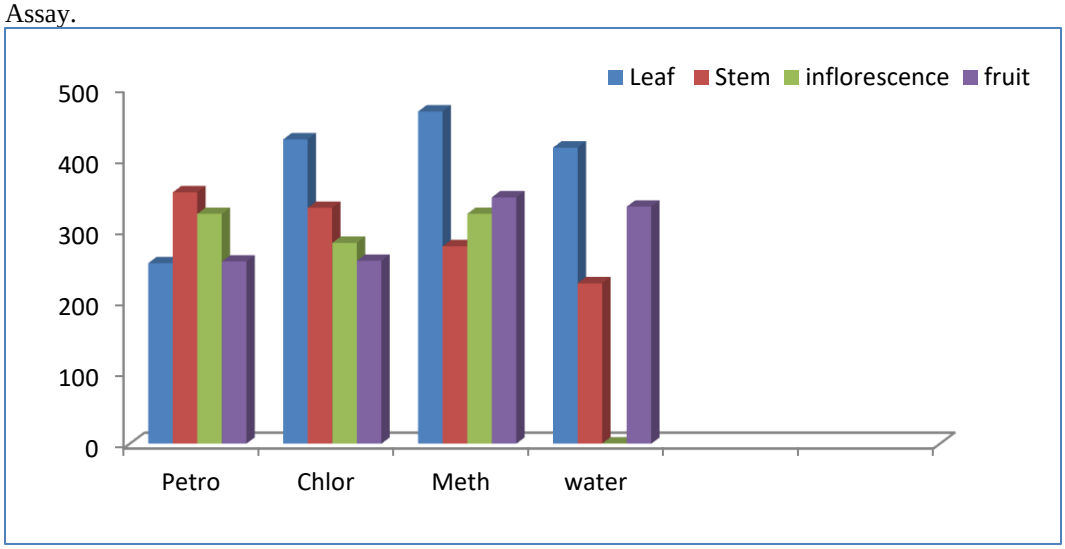

Fig. 9. Antioxidant activity of various extracts of *B. papillosa* through Total Phenolic Assay

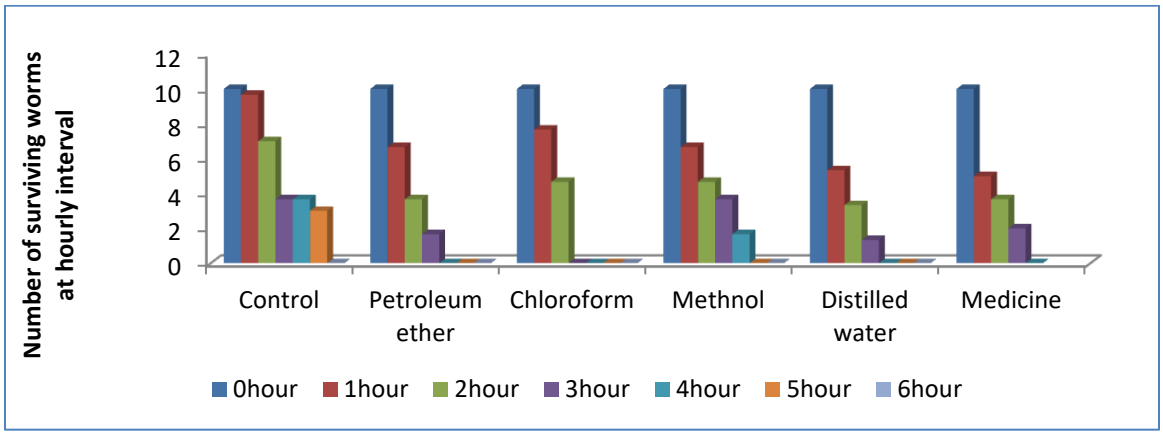

Fig. 10. *In vitro* Comparison of Anthelmintic Activity of different treatments: Leaf extracts of *B. papillosa* in various Solvents, Standard Drug (Levamisole) and Control (PBS+DMSO)

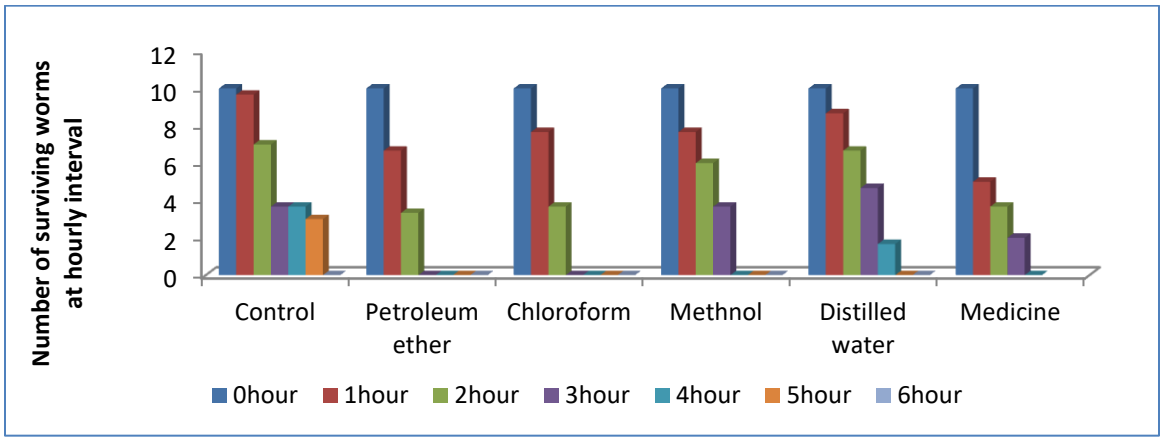

Fig. 11. *In vitro* Comparison of Anthelmintic Activity of different treatments: Stem extracts of *B. papillosa* in various Solvents, Standard Drug (Levamisole) and Control (PBS+DMSO)

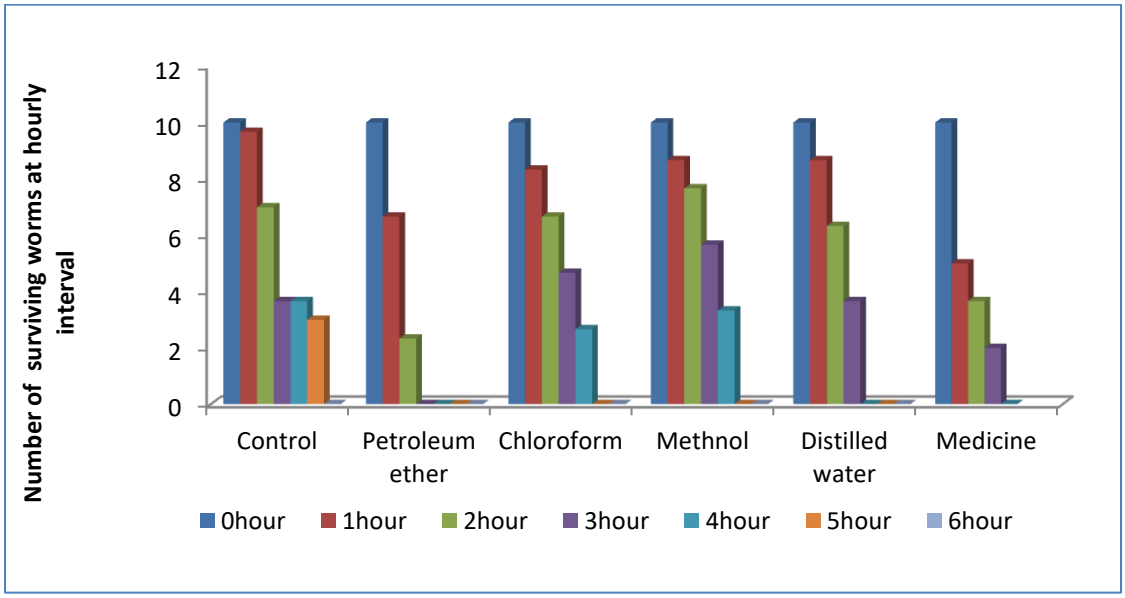

Fig. 12. *In vitro* Comparison of Anthelmintic Activity of different treatments: Inflorescence extracts of *B. papillosa* in various Solvents, Standard Drug (Levamisole) and Control (PBS+DMSO)**.**

DISCUSSION

The use of herbs and medicinal plants as the first medicine is a universal phenomenon. As plants usually have healing property, due to this every culture on earth depends on vast variety of natural plants whether through written or oral tradition. All drugs that were extracted from plant in the past have some therapeutic action. That's why medicinal plants can be used for both culinary or medicinal purpose.

The results indicated that all the plant fractions were antimicrobial in nature. Some of plants showed very high antimicrobial potential against bacteria and fungi, while some of them were comparatively less antimicrobial in nature. Rios and Recio (2005) carried out somewhat related work on antimicrobial activity and recorded the results relating to the results obtained in the present work.

The inflorescence distilled water extract of *B. papillosa* showed the maximum zone of inhibition (42 ± 0.577^{a}) against *S. aureus*, and it may be due to the polarity of the solvent. The work done by Gamboe *et al*. (2008) supports these results as they studied the antimicrobial activity of 74 extracts against both Gram positive and Gram negative bacteria and recorded similar results. Arora and Kaur (2007) also noticed the same findings from their antibacterial work.

Funtogawa *et al.* (2004) investigated antibacterial potential of medicinal plants and isolated the terpenoids and phenols from them. Fabry *et al*. (1998) reported that the plant extracts with low MIC values may serve as sources for compounds with therapeutic potency and the medicinal plants should be considered as antibacterial supplement towards the development of new therapeutic agents.

Inflorescence methanol, leaf distilled water and stem distilled water extracts of *Buxus papillosa* were more closer to α-Tocopherol with the values 85.3 ± 3.78, 82.8 ± 1.37 and 84 ± 1.51 respectively. In the same way fruit methanol extract showed its closeness with BHT with the value of 74.4 ± 2.04. These are actually the percentage DPPH values which are showing their close relation with the standard samples. The work done by Vimal *et al*. (2009) is directly related with the current study and indicates therapeutic potential of plants due to their high free-radical scavenging activity. Bajpai *et al*. (2005) recognized different plant sources as good antioxidants because of their total phenolic contents and high percentage scavenging activity present in them.

In the same way the Inflorescence methanol extract of *B. papillosa* showed the closest and nearest value with α-Tocopherol that is (0.53 ± 0.03). Almost all the extracts of *Ficus palmata* proved very closer to α-Tocopherol when compared. Cano and Amao (2005) analyzed different species and evaluated the antioxidant capacity and total phenolic contents and proved the plants very strong antioxidants. Stanojevic *et al.* (2009) determined the antioxidant activity. The phenolic contents and the high values of phenols showed that these plants containing specific compounds are intensely active antioxidants.

In different studies it has been observed that naturally occurring antioxidant compounds have been identified as free radicals or active oxygen scavengers from the plant sources. Hence interest has been increased significantly to exploit natural resources having antioxidant activity to substitute synthetic antioxidants, which are being constrained due to their side effects.

Among the extracts of *B. papillosa*, the stem chloroform extract showed the highest number of phenolic contents with the value 363 ± 11.06 whereas stem water extract as the weakest or showing the lowest number of phenolic contents 226 ± 11.2

In case of anthelmintic activity, the living duration of the worms is being noted. As in world Helminthes infections are the most extensive of all the infections in domesticated animals like goat, sheep etc. Hence the use of different plants can be helpful for minimizing the number of helminthes and automatically the reduction in the infection problems in dairy industry. Taur *et al.* (2012) reported that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health.

Leaf extracts of *B. papillosa* chloroform is comparatively stronger than others and standard medicine as showing 3hours living duration. A somewhat similar work was done by Iqbal *et al*. (2004) and Deore and Khadabadi (2009) and analyzed the anthelmintic activity in comparison with Levamisole. The worms *Haemonchus contortus* showed their mortality rate at 6 hours post exposure. Thus it is overall concluded that these plants can be effective potential candidates for the development of new strategies in future to treat microbial infections, prevent aging by antioxidants and reduce nematicidal infections as well. It is also emphasized that the herbal remedies used in the folk medicines provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help overcome the growing problems.

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