



in vitro Antimicrobial and Antioxidant Potential of Aqueous Extract of Common Medicinal Plants.

KEYWORDS

In vitro Antimicrobial, Antioxidant and Medicinal plant extracts

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ABSTRACT Traditional uses of plants have led to the investigation of their bioactive compounds resulting in significant number of remedial properties. They can serve the purpose without any side effect that is often associated with synthetic microbials. The present work has been conducted on aqueous leaf extracts of *Stevia rebaudiana*, *Withania somnifera*, *Ocimum sanctum*, *Emblica officinalis* and *Azadirachta indica* to determine their antimicrobial activity against both gram positive and negative strains as well as their antioxidant potential. The maximum anti-microbial activity was found to be of *E. officinalis* (16 mm) followed by *W. somnifera* (14 mm), *A. indica* (12 mm) and *S. rebaudiana* (10 mm) while *O. sanctum* (8 mm) was found to be least active. The maximum hydroxyl radical scavenging activity was of *Stevia* 94% whereas *Tulsi* showed significant rise in reducing power with increase in concentration.

Introduction:

Nature has bestowed upon us very rich flora with great ec-static as well as medicinal value. The plant extracts can be administered in various forms such as raw (dry extracts), tisanes, tinctures and nebulisate depending upon the type of solvent. The plant extracts are being vastly explored for their chemotherapeutic, antimicrobial, antioxidant and antibiotic as well as many other potentials. The advancement of modern techniques have made it quite easier to identify and assess common herbs for their biological potential. The secondary metabolites of plant are source of aroma, capable of producing definite physiological actions on body (Joshi *et al.*, 2006), antimicrobial (Maragathavali *et al.*, 2012, Sharma *et al.*, 2012) and antioxidant compounds (Nahak & Sahu, 2010, Pandey & Madhuri, 2010). Hence, their identification and estimation is necessary to completely explore their biotic potential. The antimicrobial and antioxidant activity of aqueous extracts of flowering plants has been conducted in the present work.

S.no	Plant	Common name	Family
1	<i>Emblica officinalis</i>	Amla	Phyl-lantheaceae
2	<i>Withania somnifera</i>	Ashwagandha	Solanaceae
3	<i>Azadirachta indica</i>	Neem	Meliaceae
4	<i>Stevia rebaudiana</i>	Stevia	Astraceae
5	<i>Ocimum sanctum</i>	Tulsi	Lamiaceae

Materials and methods:

The leaves of plants used were procured from local areas of Patiala (Punjab). The aqueous extracts were prepared as per Kumar *et al.*, (2012). A total of three clinical antibiotic resistant microorganisms including two Gram positive *Bacillus cereus* (MTCC 5521), *Nocardia asteroides* (MTCC 274) and one Gram negative bacterial strain *Escherichia coli* (MTCC 443) were procured from IMTECH Chandigarh. The antimicrobial assay was determined by agar well diffusion method as per Bhatt *et al.*, (2012) using DMSO as negative control, diameter of zone of inhibition (ZI) was measured and minimum inhibition concentration (MIC) was determined as per method of Eloff *et al.*, (1998). *In vitro* antioxidant activity was done by hydroxyl radical scavenging (HRS) activity as per Singh *et al.*, (2012) and for estimation of reducing power essay method of Oyaizu *et al.*, (1986) was followed. The hydroxyl radical scavenging was calculated as per Tarwadi & Agte, 2007 while

percentage activity by using formula, as $100 \times (\text{Absorbance of blank} - \text{Absorbance of sample}) / \text{Absorbance of blank}$ (Balaji *et al.*, 2002).

Results and Discussion:

Antimicrobial activity: The present study described maximum ZI by extracts of Amla (16 mm) followed by Ashwagandha (14 mm) against *N. asteroides*. Amla and Neem were found to be equally potent for *E. coli* (12 mm) followed by Ashwagandha and Stevia (10 mm) whereas Ashwagandha was most effective against *B. cereus* (12 mm) as compared to other extracts as depicted in figure 1. Amla was found to be most effective against all the three strains with *N. asteroides* followed by *E. coli* and *B. cereus*. The aqueous extract of Tulsi was found to be minimum effective against all the three strains i.e. *E. coli* (8 mm), *B. cereus* (6 mm) and *N. asteroides* (4 mm) respectively while Dahiya & Purkayastha (2012) reported no inhibitory effect and Vinoth *et al.*, (2012) described ethanolic extracts of Tulsi more potent to *E. coli* (24mm). Antimicrobial activity of Stevia against *E. coli* showed 10mm ZI similar to Ghosh *et al.*, (2008) while Das *et al.*, (2011) reported a 9.0 mm zone of inhibition against *E. coli*.

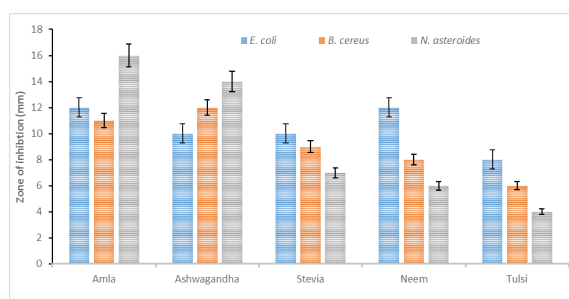


Fig 1: Zone of inhibition of various aqueous extracts against selected microbes

Minimum Inhibition Concentration (MIC): The MIC for Stevia was observed at 1:20 for *B. cereus* while the values ranging from 1:25 to 2:50 was described by Amzad *et al.*, (2010). The aqueous extract of Ashwagandha was minimum at 1:10 against *B. cereus* while it was found to be 1:30 for *E. coli* and 1:40 for *N. asteroides*. A maximum range of MIC at 1:50 for Neem against *E. coli* while minimum of 1:10 for Tulsi was found against both *B. cereus* and *N. asteroides*. MIC of Neem was observed to be 1:50 (*E. coli*), 1:30 (*B. cereus*) and 1:20 (*N. asteroides*) while Dellavalle *et al.*, (2011) reported MIC values ranging from 1:25 to 2:50 for Neem. The Tulsi

extract was found to produce significant effects against *E. coli* (1:20), *B. cereus* and *N. asteroides* (1:10) whereas, Mishra & Mishra (2011) found maximum inhibition of growth at the conc. of 0.40 to 0.78. The extracts were found to be with lowest MIC of 1:20 for *E. coli* by Tulsi, 1:10 for *B. cereus* by Ashwagandha and Tulsi, 1:10 for *N. asteroides* by Tulsi. Thus Tulsi reflects maximum inhibitory effect on all the three strains at lowest concentration.

Antioxidant activity:

Hydroxyl radical scavenging activity (HRS):

Regarding HRS activity as described in figure (2) maximum value was found for Stevia (94.2%) as compared to Amla (88%), Ashwagandha (74%), Neem (48.5%) and Tulsi (15.9%) respectively. Thus Tulsi in present study has shown least activity as compared to other tested extracts whereas Bole *et al.*, (2010) estimated quite high HRS for Tulsi 78.9% and Neem 80.6%. The antioxidant activity of aqueous and ethanolic extract was found to be increased in a dose dependent manner indicating oxidising and free radical compound in Tulsi by Gupta *et al.*, (2012). Antioxidant activity of Stevia was estimated to be 88.2% by Jahan *et al.*, (2010) which is less than the present observation 92.4%. Therefore, HRS activity Amla exhibited a higher antioxidant activity as compared to Ashwagandha which are in concordance with finding of Tarwadi & Agte (2007) who described Amla as one of the four most potent potential herb of thousands of herbs screened for superoxide scavenging.

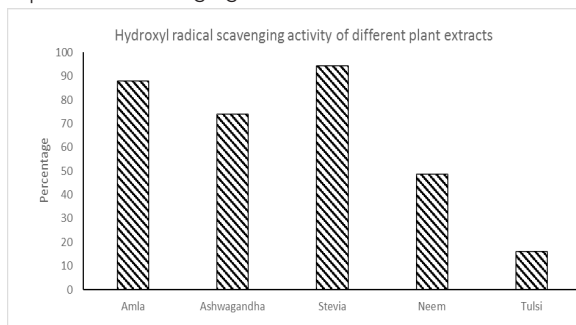


Fig 2: The percentage Hydroxyl Radical Scavenging activity of different plant extracts

Reducing power assay:

The reducing potential of various extracts has been depicted in figure (3). Maximum reducing potential is shown by Tulsi at concentration of 700 showing significant rise with increasing concentration. The reducing power of Tulsi was found to be 0.01- 0.16% similar to Bole *et al.*, (2010). The reducing power of Tulsi increases with increasing conc. of sample $p > 0.001$ and is concordant with findings of Davis, 2000 (who reported free radical damage normalized in a dose-dependent manner). The reducing power of Amla, Neem and Ashwagandha has not shown any significant change with increasing concentration. Stevia represented almost double fold rise in reducing power by raising concentration 100 to 200 where thereafter no significant rise has been estimated. Reducing power of Neem was observed to be 0.07, Amla within 0.06 to 0.07 and Ashwagandha 0.05 – 0.06 without any significant variation with increasing concentration.

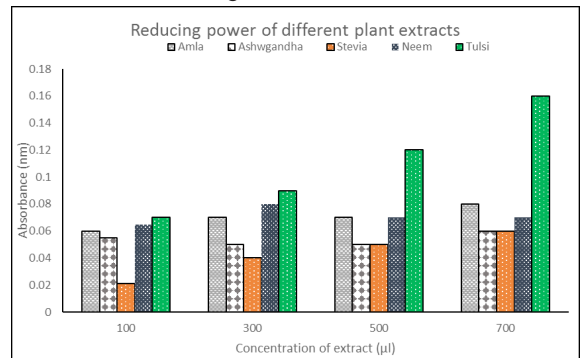


Fig 3: The Reducing power activity of the plant extracts

Conclusion:

The growing global concern in the recent years about the alarming increase in the rate of infection by antibiotic-resistant or multi-resistant microbes is leading to exploration for plants with antimicrobial activity. The present study suggest that Stevia has the best reducing potential as compared to other studied extracts and Amla has the most potent antimicrobial activity against *N. asteroides*. This study supports the use of these herbal preparations not only as the dietary supplement such as antioxidant but also as an agent to prevent or control the bacterial infections.

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