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PHYSICO-CHEMICAL PROPERTIES, TOTAL ANTIOXIDANT ACTIVITY AND IN VITRO ANTIBACTERIAL ACTIVITY OF SOLANUM ANGUIVI L. AND EMBLICA OFFICINALIS FRUIT EXTRACTS

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ABSTRACT

The present study was aimed to determine the physical and chemical properties, total phenol content, tannin content, phytochemical screening, total antioxidant activity and *in vitro* antibacterial activity of *Solanum anguivi* L.(S), *Emblica officinalis* (E) extracts and its three combinations designed to control the enzymatic browning reaction in *Solanum anguivi* L extract. *Solanum anguivi* L. (S) and *Emblica officinalis* (E) were spherical in shape, acid in nature and revealed the presence of most beneficial bioactive compounds. The combined extract of *Solanum anguivi* L. and *Emblica officinalis* at the ratio of 1:3 had higher amount of total phenol and tannin which directly enhanced the total antioxidant activity and also showed the highest resistance against various pathogenic microorganisms such as *E. coli*, *Pseudomonas aerouginosa*, *Staphylococcus aureus* and *Bacillus subtilis* when compared with individual and other combinations. Thus the combination of *Solanum anguivi* L. and *Emblica officinalis* extracts possessed high amount of phytochemicals which could be used to develop antibacterial drugs.

INTRODUCTION

Solanum anguivi L., (*S. anguivi* L.) is a flowering shrub, 3 m tall with scattering branches, often prickly, bearing small, sessile tufted hairs with 4-8 arms. The fruit is a subglobose berry 7-18 mm in diameter, smooth, green color when young and red color when ripe, usually in clusters of up to 20 fruits. The plants are consumed as vegetables and or fruits and are very rich in essential minerals and vitamins (Dentol and Nwangburuka, 2011). Fresh or dried fruits of *S. anguivi* L. are used as a traditional medicine furthermore it has been reported that they formed dietary staple food and are recommended as supplements for pregnant and lactating mothers, the young, the aged and anemic patients (Elekofehinti *et al.,* 2012). *S. anguivi* L. berries are especially characterized by their bitterness due to the presence of various phenolic compounds conferring them antioxidant properties (Denis *et al.,* 2010). The chemical changes that occur in this fruit are enzymatic browning reactions (polyphenol oxidation) which induce a pronounced loss or changes in microbiological and antioxidant qualities (Toivonen and Brummell, 2008). Thus, preservation against enzymatic browning in food during processing and storage has become an increasing priority in the food industry.

In terms of origin, antioxidant can be natural or synthetic. Although, synthetic antioxidants are effective in prevention enzymatic browning but there is league of limitations notably safety concern. However, natural antioxidants are effective and safe because they are parts of plants that man has been eating from prehistoric times. Natural antioxidants can be sourced from any part of the plants; fruit, seed, bark, flower and herb. Sourcing of antioxidants from natural means and application for treatment and management of public disease has been on the increase in recent times (Pourmorad *et al.,* 2006).

Emblica officinalis (*E. officinalis*) is one of the distributed plants in subtropical and tropical areas of India, China, Thailand and Indonesia (Liu *et al.,* 2008). Scientific studies on the plant showed that *E. officinalis* has antimalaria (Pinmai *et al.,* 2010), free radical scavenging, antioxidant, antidiabetic activity (Nampoothiri *et al.,* 2011), antibacterial activity (Ghosh *et al.,* 2008). It is also be play an important role in cure of anemia, dyspepsia, jaundice, haemorrhage and diarrhoea (Perianayagama, 2004; Baliga and Dsouza, 2011). On the other

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hand, plant showed an extensive spectrum of biologically active compounds (Liu *et al.,* 2008; Khan, 2009; Arora *et al.,* 2012; Gavatia *et al.,* 2012). *E. offiinalis* is frequently used to prevent enzyme catalysed browning reactions due to its abundant content of ascorbic acid (300-900 mg%). It holds the copper prosthetic group of polyphenol oxidase and reducing quinines back to phenols before forming dark pigments (Newman *et al.,* 2011). Thus the present investigation was aimed to integrate *E. officinalis* into *Solanum anguivi* L. extract at different ratios to prevent the enzymatic browning reaction or polyphenol oxidation and to identify the best out of three combinations according to its physico-chemical properties, screened phytochemicals, total antioxidant activity and *in vitro* antibacterial activity.

MATERIALS AND METHODS

Selection of Fruits

The matured fruits of *S. anguivi* L. and *E. officinalis* were purchased from local market at Salem, Tamil Nadu. The fruits were identified and authenticated at the Institute of Herbal Science, Plant Anatomy Research Centre, Chennai (PARC/2016/3297, PARC/2016/3296).

Chemicals

Gallic acid, tannic acid, sodium phosphate, ammonium molybdate, ascorbic acid, sulphuric acid, sodium carbonate, Folin-Ciocalteau reagent and Folin–Denis reagent of analytical grade were procured from M/s Sigma Chemical Co. Other ingredients such as nutrient agar, nutrient broth and distilled water were purchased from M/s Hi-media Chemical Co. All clinical pathogens (*E. coli*, *Pseudomonas aerouginosa*, *Staphylococcus aureus* and *Bacillus subtilis*) were isolated from medical samples and confirmed at the research laboratory of the Department of Microbiology, Periyar University, Salem, Tamil Nadu.

Fruit Extract

The fruits were washed thoroughly and wiped with dry cloth. The juices of *S. anguivi* L. Coded as S *and E. officinalis* coded as E were extracted by chopping and grinding the fruit without addition of water. The extract was filtered through 4-fold muslin cloth and the pulp free extract of each fruit and its three different combinations (10ml:30ml, 10ml:10ml, 30ml:10ml) coded as S1:E3, S1:E1 and S3:E1 were used for further analysis. Color of the fruits extract was judged visually.

Physical Properties

The physical properties of randomly selected *S. anguivi* L. and *E. officinalis* fruits such as weight (gm) recorded using the electronic balance (Parveen and Khaktar, 2015), length (mm), width (mm) and thickness (mm) by digital vernier calliper (Parveen and Khaktar, 2015), circumference (mm) by flexible non-stretchable tape, shape characteristics such as aspect ratio (Jaliliantabar *et al.,* 2013), surface area (Dash *et al.,* 2008), projected area (Ozarslan, 2002) and volume of the fruit (Ingale *et al.,* 2016) by the specified formula were determined.

Chemical Properties

The total yield of extract (Xue *et al.,* 2013), total soluble solid (Vinita *et al.,* 2012), titrable acidity and pH (Khosa *et al.,* 2011); screening of phytochemicals (Thenmozhi and Sivaraj, 2011), total phenol (Ting *et al.,* 2007) and tannin content (Vasundhara *et al.,* 2013) were measured as chemical properties of each fresh fruit (*S. anguivi* L. and *E. officinalis*) extracts and its three combinations.

Total Antioxidant Activity

The total antioxidant activity of each fresh fruit (*S. anguivi* L. and *E. officinalis*) extracts and its three combinations were evaluated by the method of Luqman *et al* (2009). The fruit extracts (0.5ml) was mixed with phosphomolybdenum using ascorbic acid as the standard in 1 ml of reagent (3.3ml sulphuric acid, 335mg sodium phosphate and 78.416 mg ammonium molybdate in 100ml of distilled water). The sample mixtures were incubated in a boiling water bath at 95°C for 90 min. The absorbance of the samples was measured at 695 nm.

Antibacterial Activity

Antibacterial activity of each fresh fruit (*S. anguivi* L. and *E. officinalis*) extracts and its three combinations were evaluated by the method of Pesaramelli *et al.* (2012). Mueller Hinton agar medium was poured into the petriplates aseptically and allowed to solidify. The lawns of the test bacterial strains were prepared by inoculation of 24 hrs culture of bacterial strains by spread plate method. Petriplates were allowed to remain for few minutes and the excess of inoculums were removed aseptically using micropipettes. Wells were made with the help of sterile cork borer (6mm) and the agar blocks were removed aseptically with sterile forceps and then the crude extracts were added in 30 µl, 60 µl and 90 µl concentrations. The test plates were incubated aerobically at 37^{o}C depending on the incubation time required for a visible growth. Control wells containing sterile distilled water (negative control) were also incubated. After incubation, the results were recorded, as the presence or absence of inhibition zone. The inhibitory zone (ZOI-Zone of inhibition) around the well indicates the absence of bacterial growth that shows positive result and the absence of zone indicates the negative result. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993). The diameters of the zones of inhibition (ZOI) were measured in millimetres.

Statistical Analysis

Physical parameters of the extracts were obtained as average of 10 determinants; chemical properties, total antioxidant activity and total antibacterial activity were the average of three determinants. The significant difference in determined properties of each fresh fruit and combined extracts of *S. anguivi* L. and *E. officinalis* was investigated by the Analysis of Variance with LSD as post hoc comparison test and correlation (Pearson Correlation) using SPSS 17.0 version. The data in table were expressed as mean ± SD.

RESULTS AND DISCUSSION

Physical Properties

Knowledge of the physico-chemical properties of food is fundamental in analysing the characteristics of food during its processing. The study of these food properties and their responses to process conditions are necessary because they influence the treatment received during the processing and also

because they are good indicators of other properties and qualities of food (Mir *et al.,* 1993). The weight (Table 1) of the *S. anguivi* L. was greater than the average weight (0.25g-0.38g) described by Daramola (2015) for Nigerian variety. The fruit weight of *E. officinalis* was comparably greater than Desi variety (14.27±0.03g); less than the fruit of Banarasi (49.97±0.61g); Chakaiya (34.96±1.37); Kanchan (37.36±0.45g); NA-7 (39.05±0.74) varieties studied by Parveen and Khatkar (2015). The fruit weight was also found to be less than the range (37.22-42g) by Anonymous (1988); Ganavhar *et al.* (2012). The difference in weight could be explained by geographical varietal difference.

Table 1 Physical Parameters of *S. anguivi* L. and *E. officinalis*

Parameters	*S.anguivi L.*	*E.officinalis*
Weight (g)	0.95±0.2	20.80±0.4**
Length (mm)	1.21±0.09	3.28±0.23**
Width (mm)	1.3±0.07	3.32±0.23**
Thickness (mm)	1.27±0.09	3.25±0.16**
Aspect ratio %	1.08±0.1	1.01±0.05
Projected area (mm^2)	1.23±0.10	8.58±1.11**
Volume (mm^3)	1.18.24	19.54±4.06**
Surface area (mm^2)	13.31±0.68	56.09±4.36**
Geometric (mm)	4.24±0.22	17.86±1.39**

The values are mean± SD of determinations made in triplicates. ** significantly different at p<0.01.

While considering the equal three semi axes (length, width and thickness) (Table 1) of *S. anguivi* L. and *E. officinalis*, the fruit was spherical in shape with geometric mean diameter of 178.9 mm and 424 mm respectively. Parveen and Khatkar (2015) described the shape of *E. officinalis* as round to oblate. The length of *E. officinalis* was similar to the length of Chakaiya variety (32.18±0.2 mm) (Parveen and Khatkar, 2015). The length and width of *E. officinalis* fruit was within the range (length 25.12 – 41.10 mm; width 29.38 – 44.85 mm) described by Ram *et al.* (1983); Kalra *et al.* (1988); Goyal *et al.* (2007); Parveen and Khatkar (2015). Similar observation was also noted by Poonam (2009) (28.88 g weight; 33 mm length; 38 mm width). The length, width and thickness of *S. anguivi* L. also revealed spherical nature of the fruit and 70% smaller than *E. officinalis*. The geometric mean diameter obtained can be used to determine the volume and sphericity, are of atmost important in designing of hooper to handle the fruit. The low aspect ratio of *S. anguivi* L. (1.08%) than *E. officinalis* of (1.01%) signifies greater tendency of *S. anguivi* L. to roll on a surface. The projected area suggests the moisture absorption characteristics during storage; lower surface area means less area for moisture absorption during storage (Abano and Amoah, 2011).

Colour of the Extract

The fruit extracts and its combined extracts were visually assessed for colour reveals *S. anguivi* L. was thick green in color initially and it changed into blackish color due to the reaction between the oxygen and polyphenol present in the fruit cells; *E. officinalis* fruit extract was thick cream and pista green in color in all the combined extracts which were changed slowly into thick green except S1:E3 extract due to maximum ratio of *E. officinalis* act as a scavenger for removing the molecular oxygen from the reactions catalyzed by polyphenol oxidation (McEvily *et al.,* 1992). However, there was no color change even an hour in S1:E3 ratio extract and it confirmed the effect of vitamin C as antioxidant nutrient on prevention of enzymatic browning reaction.

Chemical Properties

Chemical properties (Table 2) of the *S. anguivi* L. and *E. officinalis* fruit and its combined extracts revealed that 40 % of extract was obtained from *S. anguivi* L. fruit and 60.67 % in *E. officinalis* fruit which was comparatively lower than the yield of extract reported by Madhavi and Pravallika (2015) (83.53%). The yield of extract might be influenced by agro-climatic conditions. The yield of three combined extracts (S1:E3, S1:E1 and S3:E1) were 54.33%, 50.33% and 40.33% respectively and revealed the significant ($p<0.05$) influence of *S. anguivi* L. Total soluble solid of *S. anguivi* L. and *E. officinalis* (Table 2) and its three combinations were obtained between the range of 11.16° to 15.06° Brix which was higher than described by Priya and Khatkar (2013) as 10.93° Brix in *E. officinalis*. The acidity of the *S. anguivi* L. extract was ranged from 0.2 - 0.25 with the pH of 5.84 - 5.85 which was similar with the data reported by Frazier and Westhoff (1988), the range was between 4 and 6 in vegetable juices. The acidity and pH of *E. officinalis* extract was observed as in the ranged of 2.50 - 2.52 and 2.86 - 2.87 respectively; the observed data was in agreement with values reported by Sasikumar (2013) (2.6 acidity; 3.1 pH); Karpagavalli *et al.* (2014) (2.35 acidity; 2.10 pH); Parveen and Khatkar (2015) (2.7 to 2.88 pH in five different varieties). These results suggest that the *S. anguivi* L. was a low acid fruit and *E. officinalis* was a high acid fruit. The result on pH and TSS of *E. officinalis* was in agreement with the data reported by Singh *et al.* (1987); Barthakur and Arnold (1991); Ghorai and Sethi (1996); Pragati and Dhawan (2003); Singh *et al.* (2006); Goyal *et al.* (2008) and Garg (2010).

Table 2 Chemical Parameters of *E. Officinalis* and *S. anguivi* L.

Parameters	*S. anguivi* L.	*E.officinalis*	S1:E3	S1:E1	S3:E1
Juice content (ml)	40±1^{d}	60.66±0.5^{a}	54.33±0.5^{b}	50.33±0.5^{c}	40.33±0.5^{d}
TSS (Brix Value)	14.06±0.1^{b}	15.06±0.1^{a}	13.6±0.1^{c}	12.4±0.1^{d}	11.16±0.2^{e}
pH	5.84±0.005^{a}	2.86±0.005^{e}	3.15±0^{d}	3.30±0.005^{c}	3.80±0.01^{b}
Acidity	0.23±0.02^{e}	2.52±0.03^{a}	1.27±0.01^{b}	1.01±0.01^{c}	0.62±0.02^{d}

The values are the average of four determinants. a,b,c,d,e,f alphabets indicates the significant mean difference suggested by LSD test.

Phytochemical Assessment

The result of qualitative phytochemical screening of each fresh fruit extracts and its three combined extracts of *S. anguivi* L. and *E. officinalis* (Table 3) predicted that the presence of most beneficial bioactive components (phenols, tannin, flavonoids, saponin, glycosides, carbohydrate, protein, steroid, and alkaloids) exert a beneficial action on immune system (Manjulika *et al.,* 2015) and plays a vital role in the prevention of neuronal and cardiovascular illnesses, cancer and diabetes due to their antioxidant property (Du *et al.,* 2004 and Konczak *et al.,* 2004). Saponin was absent in *E. officinalis* as previously reported by Nikhil *et al.* (2013). In addition to that coumarin and anthocyanin were absent in *S. anguivi* L. extract, but the three combined extracts revealed the presence of these compounds. Sundari *et al.* (2013) also revealed the presence of alkaloids, flavonoids, saponins, indoles and steroids in Solanum species.

Table 3 Qualitative analysis of Phytochemicals

Parameters	Fruit Extracts				
	S. anguivi L.	*E. officinalis*	S1:E3	S1:E1	S3:E1
Phenols	+	+	+	+	+
Tannins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Saponins	+	-	+	+	+
Glycosides	+	+	+	+	+
Carbohydrate	+	+	+	+	+
Protein	+	+	+	+	+
Steroid	+	+	+	+	+
Alkaloids	+	+	+	+	+
Anthocyanin	-	+	+	+	+
Coumarins	-	+	+	+	+

(+) Present; (-) Absent

Total Phenol

The total phenol content as gallic acid equivalent (Table 4) of *S. anguivi* L. extract was significantly ($p<0.01$) higher (38.35 mg/g) than *E. officinalis* extract (25.06 mg/g) and the value was closure to the study of Daramola (2015) in *S. anguivi* L. and to the result of Parveen and Khatkar (2015) in *E. officinalis*. The results were comparable with data reported by Mehta and Tomar (1979); Pragati and Dhawan (2003) and Mishra *et al.* (2009). The total phenol content of three combined extracts revealed that the majority of phenolic constituents are localized in *S. anguivi* L. extract.

Table 4 Total Antioxidant activity, Phenol and Tannin of *S. anguivi* L. *and E. officinalis* fruit extract

Parameters	*E. officinalis*	*S. anguivi* L.	S1:E3	S1:E1	S3:E1
Antioxidant activity (mg/g)	40.1 ± 0.01^{a}	18.26 ± 0.2^{e}	36.17 ± 0.2^{b}	30.11 ± 0.03^{c}	24.43 ± 0.1^{d}
Phenol (mg/g)	25.06 ± 0.1^{e}	38.35 ± 0.2^{a}	26.27 ± 0.4^{d}	28.8 ± 0.1^{c}	32.02 ± 0.03^{b}
Tannin (mg/g)	29.2 ± 0.2^{e}	48.33 ± 0.2^{a}	31.2 ± 0.2^{d}	39.30 ± 0.08^{c}	40.03 ± 0.05^{b}

The values are the average of four determinants. a,b,c,d,e,f alphabets indicates the significant mean difference suggested by LSD test.

Total Tannin

Tannins are the polyphenolic compounds that have direct antioxidant activity, especially the scavenging properties (Kumar and Ragunathan, 2013). Tannin content (Table 4) was also significantly ($p<0.01$) high (48.33 mg/g) in *S. anguivi* L. extract. The average tannin content of *E. officinalis* (29.2 mg/g) was literally higher value than the value reported by Meena *et al.* (2010) (13 mg/g). The tannin content of combined extracts reflected the intensity of tannin in *S. anguivi* L.

Total Antioxidant Activity

Total Antioxidant activity (Table 4) against ascorbic acid of *E. officinalis* extract (40.1 mg/g) was significantly ($p<0.01$) greater than *S. anguivi* L. extract (18.26 mg/g) followed by S1:E3 extract (36.17 mg/g) than the other two combined extracts which could reveal the level of *E. officinalis* inclusion. Potent antioxidant properties are observed in aonla due to high ascorbic acid and polyphenol content that is credited with prevention of the oxidation of ascorbic acid (Goyal *et al.*, 2008). Fruit tissues contain high levels of phenolics, upon cutting they are quickly oxidized by enzymes, resulting as brown discoloration of the flesh (Adams and Brown 2007). These changes induce a pronounced loss of the microbiological and antioxidant qualities (Lindley, 1998) which could be quoted as the reason for low antioxidant activity of *S. anguivi* L. extract.

Total phenol and total tannin content of all studied extracts revealed an significant correlation with total antioxidant activity respectively (phenol vs total antioxidant of *Solanum* r = 0.70*; *Emblica* r = 0.95*; S1:E3 r = 0.51*; S1:E1 r = 0.96*; S3:E1 r – 0.75* and tannin vs antioxidant of *Solanum* r = 0.25*; *Emblica* r = 0.98*; S1:E3 r = 0.6*; S1:E1 r = 0.67*; S3:E1 r – 0.94*).

In vitro Antibacterial Activity

The *in vitro* antibacterial activity of each fresh fruit extracts and its three combined extracts of *S. anguivi* L. and *E. officinalis* was done by agar well diffusion method against the test bacterial cultures and their activity potentials were quantitatively evaluated by the measurement of inhibition zones and zone diameters (Table 5 and Figure 1). *E. officinalis* extract had greater potential as an antibacterial agent against the tested bacterial cultures. It was found a regular increase in the zone of inhibition size along with the concentration of extracts in all bacterial strains. Nevertheless, there was no inhibition zone was observed in *S. anguivi* L. extract that might be caused by the continuous action of polyphenol oxidase (PPO) on phenolic compounds which were released during the process of extraction (*called enzymatic browning*). *Solanaceae*, is one of the vegetables mostly affected by PPO, due to the extensive chlorogenic acid content, the main substrate for activating these biochemical reactions (Morishita and Ohnishi, 2001; Kwak and Lim, 2005).

Table 5 Antimicrobial effects of juice extract of *S. anguivi* L. and *E. officinalis* measured by zone of inhibition (mm)

Sample	Gram Negative						Gram Positive					
	E. Coli			*Pseudomonas aeruginosa*			*Staphylococcus*			*Bacillus Subtilis*		
	30µl	60µl	90µl	30µl	60µl	90µl	30µl	60µl	90µl	30µl	60µl	90µl
E. officinalis	4	6	8	17	19	21	12	17	21	13	15	16
S. anguivi L.	-	-	-	-	-	-	-	-	-	-	-	-
S1:E3	22	27	22	28	29	17	19	23	28	29	31	19
E1:E1	20	22	15	19	23	12	19	21	22	25	29	18
S3:E1	16	25	10	15	21	10	12	16	11	14	17	10

-Sign indicates the absence of zones

The other three combined extracts showed highly significant inhibitory activity against the studied human pathogenic bacteria; S1:E3 extract was more potential to inhibit the microorganisms. In the combination of S1:E1 and S3:E1, the inhibition zone was decreased respectively, it might be due to enzymatic browning reaction in *S. anguivi* L. Thus, the *S. anguivi* L. combined with *E. officinalis* at 1:3 ratio revealed greater potential to fight against the human pathogens.

The diameter of inhibition zone was increased with increase in the concentration of *E. officinalis* extract; but in combined extracts, the 90 µl level reported reduced zone of inhibition against *E. coli*, *Pseudomonas aerouginosa* and *Bacillus subtilis* except for *Staphylococcus aureus*.

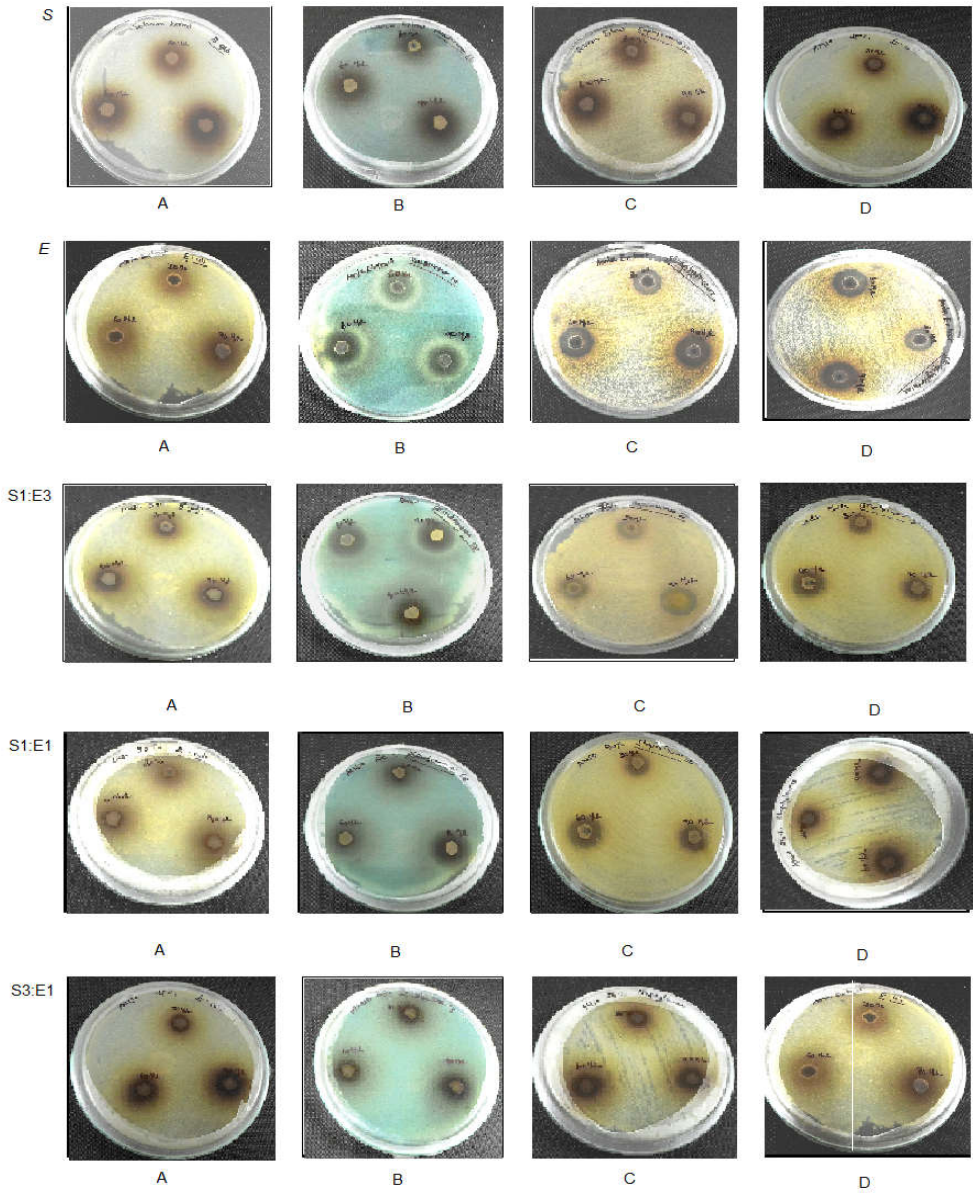

Figure 1 Antibacterial activity of *S. anguivi* L. and *E.officinalis* extract

A - *E.coli* sp. B - *Pseudomonas* sp. C - *Staphylococcus* sp. D – *Basicillus* sp.

The lowest inhibition zone ranged from 4-8 mm was observed in *E. officinalis* extract against *E. Coli* and the highest inhibition zone was detected at 28-31 mm in the S1:E3 extract against *Bacillus Subtilis*. Maryam *et al.* (2013) also tested the antibacterial activity of *E. officinalis* powder against four strains of bacteria namely *E.coli, S.aureus, Pseudomonas fluorescens* and *Bacillus subtilis* and showed similar maximum zone of inhibition against *Bacillus subtilis* and lowest against *E.coli.* He also stated that the ZOI values of Amla juice powder are dose dependent; ZOI values increased as the concentration increased. Accordingly, *E. officinalis* had greater potential as antimicrobial compounds and can be used to treat infectious diseases caused by resistant microorganisms.

CONCLUSION

Thus the present investigation revealed the combined extract of *Solanum anguivi* L. and *E. officinalis* at 1:3 ratio could potentially be a rich source of natural antioxidants and could be developed into functional foods or drug or dietary supplements for the prevention and treatment of diseases caused by oxidative stress. India is a mega diversity nation, rich in medicinal plants that have been used from time immemorial as remedies for various infections. In the future, the specific components with high antioxidant capacities in these combinations of wild fruits should be isolated and identified and explored for their health effects against oxidative stress.

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