Int J Pharm Bio Sci 2015 July; 6(3): (B) 812 - 823

Research Article **Biochemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

COMPARATIVE STUDIES ON ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF GRAPE AND PAPAYA SEED EXTRACTS USING *IN-VITRO* STUDIES

FAISAL RS[1], RAJU CV*[1], LAKSHMISHA IP[1], RAJKUMAR RS[1] AND SABA KK [2]

All India Coordinated Research Project on Post-Harvest Technology
[1] College of Fisheries, KVAFSU, Mangalore-575 001, Karnataka, India
[2]Faculty of Fisheries, SKUAST-K Jammu and Kashmir, India

ABSTRACT

In the present study, antioxidant and antimicrobial activities of Grape seed extract (GSE) and Papaya seed extract (PSE) were investigated by using different *in-vitro* models *viz.*, DPPH radical activity, ABTS scavenging activity, metal chelation, ferric reducing power, linoleic acid model and antimicrobial activity by disc diffusion method. The GSE contained four times higher phenolic and six times higher total flavonoid content compared to PSE. The extracts showed dose dependent ferric reducing ability with a very good correlation to total phenolic content (r^2 = 0.96). The linoleic acid model system with 5 days of incubation showed that the addition of GSE and PSE to the reaction mixture inhibited peroxidation by 81.2 and 61.5 % respectively. GSE showed higher antimicrobial activity on gram-positive strains compared to PSE. These findings suggest that, the GSE is a cheap and promising source of potent *in-vitro* antioxidant and antimicrobial activities.

KEY WORDS: Antimicrobial activity, Grape seed extract, Lipid oxidation, Linoleic acid model system, Papaya seed extract

RAJU CV
All India Coordinated Research Project on Post-Harvest Technology, College of Fisheries, KVAFSU, Mangalore-575 001, Karnataka, India

***Corresponding author**

INTRODUCTION

Preservation of spoilage and shelf-life extension of food products will always remain an important goal to the food industry. The auto-oxidation and microbial spoilage is a major problem as it leads to the deterioration of quality of food products and reduction in their nutritional value. Lipid oxidation is one of the serious problems for the food industry because of its subsequent development of undesirable off-flavors, odours, dark colors and potentially toxic reaction products in foods[20]. Oxidation of lipids initates other changes in the food system which affect nutritional quality, wholesomeness, safety, color, flavor and texture[32]. To retard such a quality loss, synthetic antioxidants have been used to decrease lipid oxidation during processing of food. However, the use of synthetic antioxidants has raised questions regarding safety and toxicity[6].Therefore, the importance of replacing synthetic antioxidants with natural compounds has increased greatly. In this context, the extracts of plant sources containing phenolic compounds have gained considerable attention as natural antioxidants and antimicrobial agents and have formed the basis of many applications including raw and processed food preservation[14]. Plant phenolic compounds comprise a great diversity such as flavonoids and several classes of non-flavonoids. In general, phenolic compounds play a role as antioxidants through different mechanisms of action, such as scavenging of free radicals, chelation of transition metals, quenching of reactive oxygen species[8]. Therefore, the present study is aimed to investigate antioxidant, antimicrobial activity and inhibitory effects of grape and papaya seed extracts on lipid oxidation in model systems.

MATERIALS AND METHODS

i. Chemicals

2,2-azino-bis (3-thylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS),6-hydroxy-2,5,7,8-tetram-ethylchroman-2 carboxylic acid (Trolox), 2,2-diphenyl-1-picryl hydrazyl (DPPH), Linoleic acid, potassium persulphate, Aluminum chloride,catechin, potassium ferric cyanide,Ferric chloride, Ferrozine were purchased from Sigma Chemicals Co. (St. Louis,MO,USA). All solvents used were of analytical grade and obtained from E-Merck, Mumbai, India. and chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

ii. Collection and Preparation of extracts

Grapes seeds (*Vitis vinifera*) were collected from local juice centers while papaya seeds (*Carica papaya*) were purchased from local juice processing center in Mangalore, Karnataka, India during the period of April to May. The samples were washed and cleaned with water and dried in hot air oven at 50 °C for 72 h. Dried grape and papaya seeds were ground to fine powder and stored in separate screw cap bottles before extraction. Dried powder of grape and papaya seed was used for extraction of bioactive compounds. One hundred grams of each ground material were defatted by shaking three times with four volumes of petroleum ether in a rotary shaker (Orbitek Scigenics, India) for 1 h. The residues obtained after filtration was dried overnight under a hood until all traces of petroleum ether were removed. The dried residues from each material were extracted three times with four volumes of 90% ethanol by shaking for 1 h and filtered by using filter paper. The combined filtrates from each material were concentrated in a rotary evaporator (Rotavap PBU-6D, India) and placed under a hood to remove the residual ethanol. The obtained aqueous extracts were frozen overnight and freeze-dried at - 60 °C (ModulyoD Freeze dryer 230, USA). The freeze- dried extracts were stored in air-tight containers at 5 °C until used for the determination of antioxidant and antimicrobial activity.

iii. Bacterial Cultures

Bacterial cultures namely *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2688), *Bacillus subtilis* (NCIM 2063), *Salmonella typhi* (NCIM 2501) *and*

Pseudomonas fluroscens (NCIM 2099) were procured from National chemical laboratory, Pune. India. The above cultures were grown in nutrient agar media (Hi Media, Mumbai, India) at 37 °C. Each bacterial strain was transferred from slants stored at 4-5 °C to 10 ml nutrient broth and cultivated at 37 °C for 24 h. Pre-culture was prepared by transferring 1 ml of this culture to 9 ml nutrient broth and cultivated for 48 h.

iv. Estimation of total phenolic

The total phenolic content of the grape seed extract (GSE) and papaya seed extract (PSE) was determined by the Folin-Ciocalteu method[18]. The sample of each extract solution (200 µL) was transferred to a test tube and then mixed thoroughly with 1 mL of Folin-Ciocalteu reagent. After mixing for 3 mins, 0.8 ml of 7.5 % (w/v) sodium carbonate was added. The mixtures were agitated with a vortex mixer and then allowed to stand in the dark for a further 30 mm, after which they were centrifuged at 3300 g for 5 mins at room temperature. The absorbance of extracts and a prepared blank were measured at 765 nm. The measurements were compared to a standard curve prepared by using gallic acid solution of concentration 20-100 mg/L and expressed as milligrams of gallic acid equivalents (GAE) per gram extract, which was determined from known concentrations of gallic acid standard.

v. Estimation of total flavanoid content

Total flavonoid content of the sample extracts were measured with an Aluminum chloride colorimetric assay[22]. An aliquot (1ml) of extract or standard solution of (+) catechin (20, 40, 60, 80, 100 mg/l) was added to a 10ml volumetric flask, containing 4 ml of distilled and deionized water. To the flask was added 0.3ml 5% $NaNO_2$. After 5 mins, 0.3ml 10% $AlCl_3$ was added. At the sixth minute, 2ml 1M NaOH was added and the total volume was made upto 10 ml with double distilled water. The solution was mixed well and the absorbance was measured against a reagent blank at 510nm with UV-VIS spectrophotometer. Total flavanoid content of the GSE and PSE were expressed as milligram of (+) catechin equivalent per 100 gram dry mass (mg CE /g dw).

vi. Radical scavenging activity using DPPH• method

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of extracts was determined[35]. A known volume of 1.5 ml of extract was added to 1.5 ml of 0.1 mM DPPH solution prepared in 99.5 % ethanol and thoroughly mixed by using cyclomixer at high speed. The mixture was stored in dark at room temperature for 30 min. After the incubation, the absorbance was measured at 517 nm using UV-VIS double beam spectrophotometer (UV-VIS Spectrophotometer, LaboMed, Inc., UK.). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. DPPH radical-scavenging activity was calculated and expressed in terms of percentage of DPPH free radicals scavenged using the following formula

$$\text{DPPH radical scavenging activity } (\%) = 1 - \frac{\text{Abs}_{\text{Sample}}}{\text{Abs}_{\text{Control}}} \text{ X } 100$$

vii. Radical scavenging activity using ABTS•+ method[1]

The stock solution included 7.4 mM ABTS solution and 2.6 mM potassium persulphate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1 ml ABTS solution with 50 ml methanol in order to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using a spectrometer (UV-1601 spectrophotometer, Shimadzu, Kyoto, Japan). A fresh ABTS solution was prepared for each assay. Samples (150 µl) with a concentration range of 0.5–10 mg/l were mixed with 2850 µl of ABTS solution and the mixture was left at room temperature for 2 h in dark. The absorbance was then measured at 734 nm. A sample blank at each concentration was prepared using methanol instead of ABTS solution. A standard curve of Trolox ranging from 50 to 600 µM was prepared. The activity was expressed as µmol

Trolox equivalents (TE)/ml of phenolic compound.

viii.Reducing power assay[27]

An aliquot of 1 ml was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 °C for 30 min. After incubation, 2.5 ml of 10% (w/v) trichloroacetic acid was added. Finally, 2.5 ml of the solution from the mixture was drawn and mixed with 2.5 ml of distilled water and 0.5 ml of 0.1 % (w/v) ferric chloride solution. After 10 min of reaction time, the absorbance of the resultant solutions was measured at 700 nm using UV-VIS Spectrophotometer. Higher absorbance of the reaction mixture indicates higher reducing power.

ix. Metal chelating activity[4]

Samples (4.7 ml) with a concentration ranging from 0.5-3 mg/l were mixed with 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine. The reaction mixture was allowed to stand for 20 min at room temperature. The absorbance was then read at 562 nm. The blank was prepared in the same manner using distilled water instead of the sample. The chelating activity after the sample blank substraction was calculated as follows

Metal chelating activity (%) = $(A_{blank} - A_{sample})/A_{blank} \times 100$

Where, A_{sample} is the absorbance of the sample and A_{blank} is the absorbance of blank.

x. Determination of antioxidant activity using linoleic acid model[26]

One ml of extract, dissolved in 10 ml of 50 mM phosphate buffer (pH 7.0) was added to a solution of 0.13 ml of linoleic acid and 10 ml of 99.5% ethanol. The total volume was then adjusted to 25 ml with distilled water. The mixture was incubated in a 30 ml assay tube with a screw cap at 40 ± 1 °C for 5 days in an incubator. The dark room condition was maintained by wrapping with aluminum foil and thicker paper. The degree of oxidation of linoleic acid was measured using the ferric thiocyanate method[24]. To 0.1 ml of the reaction mixture, 4.7 ml of 75 % ethanol, 0.1 ml of 30 % ammonium thiocyanate, and 0.1 ml of 20 mM ferrous chloride solution prepared in 3.5 % HCl were added. After 3 mins of incubation, the color was measured at 500 nm. Appropriate controls were maintained using ethanol. The antioxidative capacity to inhibit the peroxide formation in linoleic acid was expressed as follows

$$\text{Lipid peroxidation inhibition } (\%) = 1 - \frac{Abs_{Sample}}{Abs_{Control}} X\,100$$

xi. Antimicrobial activity by disc diffusion method

The antibacterial activity of the GSE and PSE was performed by following agar disc diffusion method[3]. Bacterial strains were first grown on Muller Hinton medium for 18 to 24 h at 37 °C. The inoculum of the indicated bacterial strains were transferred into physiological suspension medium and adjusted to 0.5 Mac Farland turbidity standards. A sterile 10 mm-diameter filter disc impregnated with 10 µl 1000 µg per disc of each extract suspended in dimethyl sulphoxide (DMSO) was placed on the infusion agar seeded with bacteria. Then, Petri dishes were kept at 4 °C for 1 h and subsequently incubated at 37 °C for 24 h. Ampicillin (10µg) (Hi Media India) standard discs were used as positive antibiotic controls. Discs impregnated with 10 µl of pure DMSO were used as negative controls. The antibacterial activity was assessed by measuring the zone of growth inhibition surrounding the discs.

xii. Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means were determined by Duncan's multiple range test ($p<0.05$) using statistical software (Statsoft Inc., Tulsa, USA). Values expressed are means of three determinations ± standard deviation.

RESULTS AND DISCUSSION

I. Yield percentage, total phenolic and total flavonoid content of GSE and PSE

The percentage yield of extracts, total phenolic and total flavonoid contents of GSE and PSE are presented in Table 1. It was observed that, the maximum yield was obtained for the GSE. The percentage yield of both GSE and PSE were 6 and 2.75 % respectively. Total Phenolic content of GSE and PSE were expressed as Gallic acid equivalent per gram of sample. The grape seeds were observed to possess higher TPC i.e. 1.23 mg GAE/g grape seed when compared to papaya seed it was 0.32 mg GAE/g papaya seed. It was found from the study that, GSE contains four times higher phenolic content than PSE. The TPC of Norton grape was 1.8 mg GAE/g fresh grape[11]. The percentage of phenolic content of Bangalore blue grapes in acetone: water: acetic acid and methanol: water: acetic acid was found to be 46 % and 38 % respectively, which showed that the increase in the content depends upon the solvent used for extraction[15]. Other researchers have documented a slightly higher phenolic content in red wine ranging from 1.4 to 3.1 mg GAE/g fresh grape (Yi et al.1997). The quantification of TPC showed an approximately 26 times higher content in grapes than in rosemary extract[9]. The higher content of phenolics in grapes indicates a better radical scavenger which can prevent lipid oxidation in food products. The total Phenolic content was 3.32 mg GAE/ g papaya seed[21]. The total phenolic content of papaya seeds was 8 mg GAE/100 g fresh weight, which was comparatively much lower than the present study[25]. Grape and papaya seed extracts significantly varied in total flavonoid contents i.e. 1.662 ± 0.3 and 0.282 ± 0.3 respectively. It was observed that GSE had six times higher total flavonoid content when compared to PSE. The results of the present investigation was found to be similar to the findings of three grape extracts, which varied significantly in total flavonoid content ranging from 0.48 mg CE/g for the cabernet Franc clone 313 to 1.19 mg CE/g for Norton[11].The flavonoid content of the GSE used in present study was slightly higher than that of seven table grape varieties i.e. 0.13 to 0.31 mg CE/g[5]. The flavonoid content of PSE is comparatively lower to that of GSE as agreed with the previous findings[12].

II. DPPH radical scavenging activity of GSE and PSE

In the present investigation the DPPH scavenging activity of grape and papaya seed extracts at different concentration is shown in Fig 1. The activity of both the extracts increased with increase in concentration ($P < 0.05$). At the same concentration used, the descending order of DPPH radical scavenging activity of the seed extract tested was as follows: GSE > PSE. The DPPH radical activity of GSE increased upto 300 ppm and thereafter the activity did not showed any further increase ($P > 0.05$). Results suggests that BHA show highest radical scavenging activity of 97.33% compared to both the extracts, whereas GSE and PSE showed an activity of 87.02 and 61.43% respectively at 500 ppm. The high radical scavenging of GSE could be due to flavonoids that can perform scavenging action on free radicals (superoxide, hydroxyl and 1,1-diphenyl-2-picrylhydrazyl (DPPH)), metal chelating properties, reduction of hydroperoxide formation and their effects[13]. The presence of the functional group "-OH" in the structure and its position of the flavonoid molecule determine the antioxidant capacity[2]. Addition of -OH group to the flavonoid nucleus will enhance the antioxidant activity, While substitution by $-OCH_3$ groups diminishes the antioxidant activity. The other reason for higher scavenging activity of GSE is due to high amount of total phenolic content and the presence of monomeric polyphenolic compounds such as (+)- cathecin , (-) – epicathecin and epicathecin-3-o- gallate and dimeric and tetrameric procyanidins[30]. The lower scavenging activity of papaya seed extracts could be due to P-hydrobenzoic acid and vanillic acid which are simple phenolic compounds[38]. The carboxyl group of hydrobenzoic acid and methoxy group of Vanillic acid might explain negative effect on its antioxidative activity. The carboxyl group is the

electron withdrawing group which doesn't benefit the radical scavenging activity of the compound[34]. The result indicated that grape seed were the potent free radical scavengers, which reacted with radicals by donating their hydrogen and acts as primary antioxidants which can be used as a rich source of functional and antiradical compound to prevent lipid oxidation.

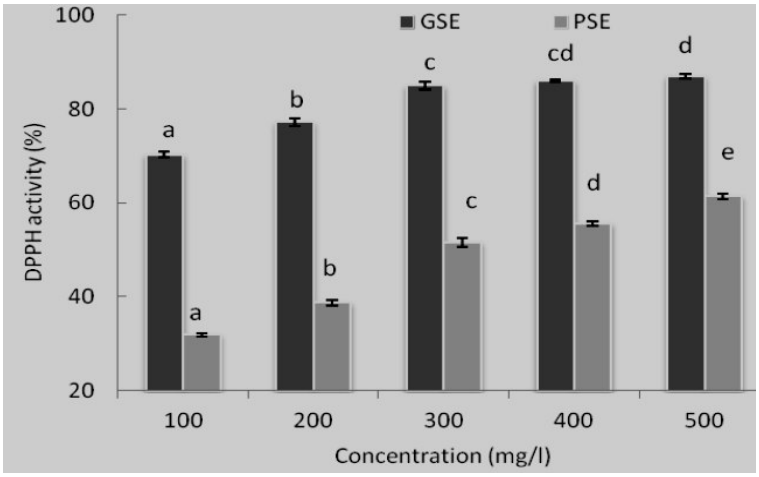

Figure 1
DPPH radical scavenging activity of GSE and PSE at various concentrations

III. ABTS radical scavenging activity of GSE and PSE

ABTS scavenging activity of GSE and PSE at different concentration is shown in Fig 2. $ABTS^{\bullet+}$ radical scavenging activity of both the extracts increased as the concentration increased ($P< 0.05$). However, the activity varied with the type of extract tested. The dose-dependent scavenging activity is shown in PSE while in case of GSE there was no significant difference at 200 - 600 mg /lt ($P>0.05$) and showed a similar trend with the results of the DPPH• assay. The low ABTS•+ scavenging activity of PSE is reported by other authors[28, 19]. The extraction of grape seed with different solvents showed varied ABTS•+ radical scavenging activity and it depends upon polarity of the solvent, the isolation procedure and the purity of the active compounds as well as test system used to evaluate the activity[23]. The effectiveness to quench the ABTS radical depends upon the number of aromatic rings in the antioxidant, nature of hydroxyl groups and molecular weight[10]. The higher TEAC value indicates that the mechanism of antioxidant action of extracts was as a hydrogen donor and it could terminate the oxidation process by converting free radicals to the stable forms. So it was more likely said that the structure and side group of phenolic compounds had the capacity to scavenge radicals and different compounds have the ability to scavenge differently. As a consequence, different assays should be conducted to verify the antioxidant activity of various compounds, in which mode of action could be different. The results strongly suggests that GSE is strong ABTS•+ radical cation inhibitor as compared to PSE.

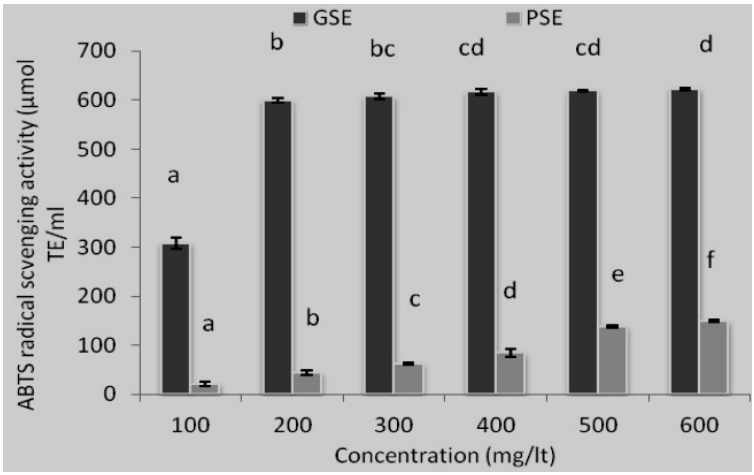

Figure 2
ABTS radical scavenging activity of GSE and PSE at various concentrations

IV. Ferric reducing antioxidant power of GSE and PSE

The reducing ability of the GSE and PSE were evaluated at different concentration (100. 200, 300, 400, and 500 µg/ml) as shown in Fig 3 and were compared to standard Butylated hydroxyl toluene (BHT) at 200 ppm. All the extracts were capable of reducing Fe^{3+} and did so in a linear dose dependent manner. Among the two extract tested, GSE showed highest ferric reducing activity at all concentration ($P < 0.05$) and showed highest reducing power at 500 µg/ml which is almost equivalent to BHT at 200 ppm., indicating that GSE could easily donate electron to Fe^{3+}, thus reducing it to Fe^{2+}. The ability of grape seed to show good reducing power is due to cathecin and epicathecin possessing the higher number of higher hydroxyl groups. The results of high reducing power of GSE were in agreement with highest DPPH and ABTS radical scavenging activity. GSE was able to reduce Fe^{3+} to Fe^{2+} and with an increase in concentration ferric reducing power increases[37]. In Contrary papaya seed extract shows least reducing power and the results were in agreement with less Phenolic content, DPPH and ABTS radical scavenging activity. Ethyl acetate extract of PSE possessed the greater reducing power than did ethanol extract fraction, *n*-butanol fraction and water fraction which is in accordance with the results of the current study[38]. The results suggests reducing power of the compound appears to be related to degree of hydroxylation and extent of conjugation in polyphenols which is seen in GSE as it contain cathecin and epicathecin which are highly hydroxylated while in case of PSE it lacks hydroxylation in its structure, which could affect in ferric reducing ability.

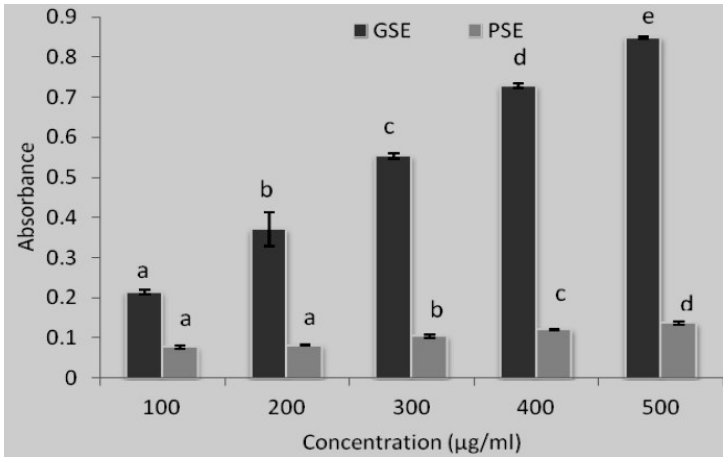

Figure 3
Ferric reducing antioxidant power of GSE and PSE at various concentration.

V. Metal chelating activity of GSE and PSE

GSE and PSE extracts were assayed for their metal chelating activity at different concentrations as depicted in Fig 4, and this activity was compared with the chelating activity of synthetic metal chelator EDTA at 0.05Mm. GSE chelated more iron than PSE ($P < 0.05$), although both extracts were less efficient than commercial chelator EDTA. Metal chelating activity of the compound depends upon number of hydroxyl groups in ortho position. The low chelating activity of PSE was possibly due to the presence of methoxy group. The chemical structures of papaya seed extract were identified as p- benzoic acid and vanillic acid[38]. The methoxy group of vanillic could interfere in metal chelating activity of PSE and that may be the reason for low metal chelating activity than GSE. Methoxy group could stabilize phenol radicals owing to its electron donating abilities but didn't contribute in chelating activities[7].

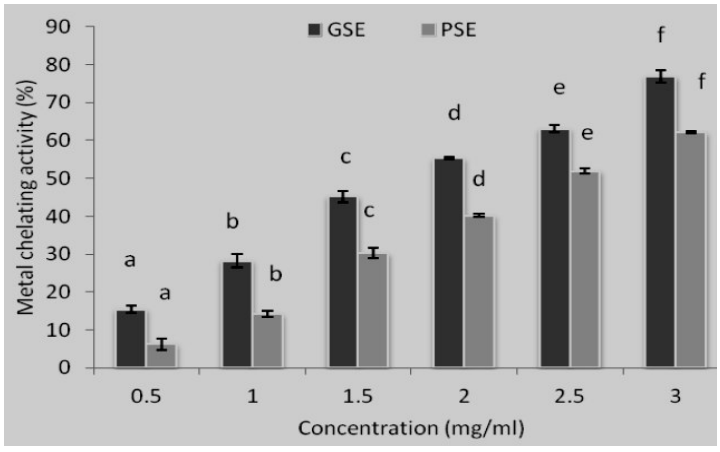

Figure 4
Metal chelating activity of GSE and PSE at various concentrations

VI. Lipid peroxidation inhibition of GSE and PSE

The inhibitory capacity of GSE and PSE against the oxidation of Linoleic acid model system was tested. As shown in Fig 5, the percentage of inhibition of lipid oxidation by grape and papaya seed was measured at three different concentrations. At the same concentration, grape seed was observed to have higher

inhibitory activity (P< 0.5) than papaya seed extracts and with the increase in concentration inhibitory activity of both the extracts increases to a maximum of 81.02 and 65 % respectively for GSE and PSE. GSE could inhibit lipid peroxidation in the mouse liver microsome model system by 70-80%[29]. Moreover, the ethanolic and water grape seed showed 80% inhibition of the Linoleic peroxidation after 100 h[16]. The mechanism involved for interference of GSE inhibition of lipid oxidation is either iron chelating activity or by scavenging of superoxide radicals, which are responsible for reduction of ferric to ferrous, catalyzed by Fenton reaction and the iron chelating activity[29].

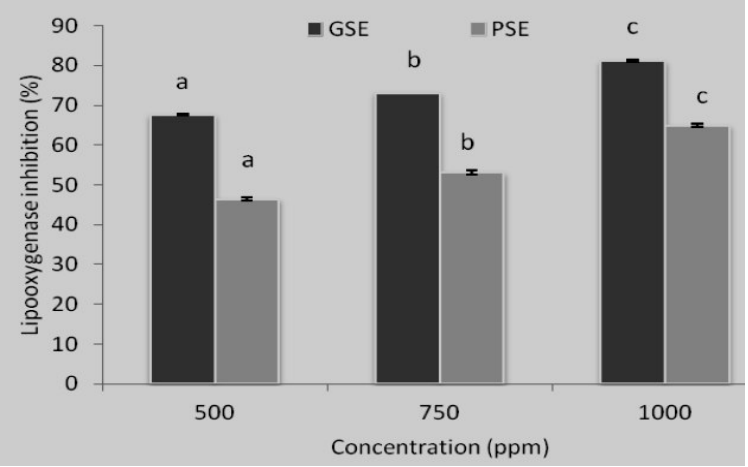

Figure 5
Inhibition of lipid oxidation in linoleic acid model system by GSE and PSE used at various concentrations

VII. Antimicrobial activity of GSE and PSE

The antibacterial activity of grape and papaya seed extracts is shown in Table 2. Among the two extracts tested against gram positive and gram negative bacteria, GSE showed higher antimicrobial activity compared with PSE. GSE was more effective against *Staphylococcus aureus* and *Bacillus subtilis* compared to gram negative bacteria. These results are in agreement with other reports[31,15]. The higher activity against the gram positive strains may be due to the fact that gram positive bacteria have less stable cell wall, which make it permeable to some antimicrobial agents. The antimicrobial properties of grape seed could be due the presence of core compounds with 3,4,5-trihydroxyphenyl found in epigallocatechin, epigallocatechin-3-ogallate and prodelphinidin, which might play an important role in their antibacterial activity[33]. The galloyl groups present in their structure of compounds present in GSE could exhibit antimicrobial activity. The mechanism of antimicrobial activity is due to outer cell membrane or cytoplasmic membrane of the bacterium is essentially composed of phospholipid bilayer and proteins and is the major site of interaction with antimicrobial compounds.

Table 1
Percentage yield of extracts, total phenolic and total flavonoid contents of GSE and PSE

Seed extracts	Yield (%)	Total phenolic content (mg GAE/g DW)	Total flavonoid content (mg CE/g DW)
GSE	6.00	1.23 ± 0.80	1.66 ± 0.30
PSE	2.75	0.31 ± 0.50	0.28 ± 0.30

Table 2
Effect of grape seed extract (GSE) and papaya seed extract (PSE) on the growth of pathogenic microorganisms

Pathogenic Microorganisms	Zone of inhibition in mm		
	Grape seed extract	Papaya seed extract	Ampicillin
S. aureus	27.04 ± 0.86	25.42 ± 1.04	55.02 ± 1.32
B. subtilis	30.03 ± 0.838	25.00 ± 0.76	51.50 ± 0.40
P. fluroscens	18.66 ± 0.251	-	26.02 ± 0.45
S. typhium	13.01 ± 0.32	15.15± 0.25	11.33 ± 0.763
E.coli	15.13 ± 0.32	17.20 ± 0.25	25.16 1.60

CONCLUSION

Potential risks and concern over the use of synthetic antioxidants and antimicrobials have renewed the interest of consumers towards natural and safe alternatives in food products. To address the need, several plant extracts are being used in the industry. Grape and papaya seed extracts have proved to be a natural source of antioxidant and antimicrobial compounds, which have the ability to improve the overall quality and extend the shelf life of food products. They can also be used in various food systems such as meat, probiotics and packaging films to increase the microbial quality and safety of the foods which spoil soon. The present study revealed that, the GSE possess higher phenolic and flavonoid content in comparison to PSE, highlighting its contribution to the antioxidative activity in the array of in-vitro assays. Moreover, GSE showed higher inhibitory activity against lipid oxidation in linoleic acid model system and a promising antimicrobial action towards *Staphylococcus aureus* and *Bacillus subtilis*. Hence the use of these extracts could offer the food processing industries a safer and alternative solution to synthetic antioxidants and antimicrobials. In continuation, the study necessitates investigations on the isolation of the compounds responsible for the activity and its mechanism of action in various foods.

ACKNOWLEDGMENT

The authors wish to express their sincere thanks to the Director of Research, KVAFSU, Bidar for continuous support to conduct this work. Authors also thank the Dean, College of Fisheries and the Head, Department of Fish Processing Technology for extending facilities to conduct this work. The financial assistance from All India Coordinated Research Project on Post-Harvest Technology, CIPHET (ICAR) Ludhiana, India, in carrying out this study is gratefully acknowledged. Joseph R Robinson and Vincent HL Lee, Ed. Controlled Drug Delivery Fundamentals and applications, 2nd Edn, Vol 29, Lippincott Williams s publisher: 555 561, (1994).

REFERENCES

1. Arnao MB, Cano A, Acosta M, The hydrophilic and lipophilic contribution to total antioxidant activity. Food. Chem., 73: 239–244, (2001).
2. Arora A, Nair MG, Strasburg GM, Structure–activity relationships for antioxidant activities of a series of flavonoids in a liposomal system. Free. Rad. Biol. Med., 24: 1355–1363, (1998).
3. Bauer AW, Kirby WMM, Sherrris JC, Antibiotic susceptibility testing by a standard single disk method. Amr. J. Clin Pathol., 45: 493-496 (1996).
4. Boyer RF, Mccleary CJ, Superoxide ion as a primary reductant in ascorbate-mediated ferritin iron release. Free. Rad. Biol. Med., 3: 389–395(1987).
5. Cantos E, Espin, JC, Tomas- Barberan FA, Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS. J. Agric. Food. Chem., 50 (20): 5691-5696 (2002).
6. Chang SS, Ostric-Matijasevic B, Hsieh OA, and Chang CL, Natural antioxidants from rosemary and sage. J Food Sci., 42:1102–1106 (1977).
7. Danilewicz JC, Review of reaction mechanisms of oxygen and proposed intermediate reduction products in wine: Central role of iron and copper. Am. J. Enol. Viticul., 54(2): 73–85.(2003).
8. Edenharder R, Grunhage D, Free radical scavenging abilities of flavonoidsas mechanism of protection against mutagenicity induced by terbutylhydroperoxide or cumene hydroxide in Salmonella typhimurium TA102. Mut. Res. Gene. Toxicol. Enviro. Muta., 540: 1-18 (2003).
9. Gibis M, Weiss J, Antioxidant capacity and inhibitory effect of grape seed and rosemary extract in marinades on the formation of hetrocyclic amines in fried beef patties. Food Chem., 134:766-774 (2012).
10. Hangerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel, TL.. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food. Chem., 46: 1887–1892,(1998)
11. Hogan S, Zhang L, Li J, Zoecklein B, Zhou K, Antioxidant properties and bioactive components of Norton (*Vitis aestivalis*) and Cabernet Franc (*Vitis vinifera*) wine grapes. J. Food. Sci. Tech., 42: 1269-1274 (2009).
12. Irondi AE, Anokam KK, Ndidi US, Effect of drying methods on the phytochemical composition and antioxidant activities of Carica papaya seed. Int. J. Biosci., 3: 154-163(2013).
13. Jacob JK, Hakimuddin F, Paliyath G, Fisher H, Antioxidant and antiproliferative activity of polyphenols in novel high-polyphenol grape lines. Food. Res. Int., 41: 419–428 (2008).
14. Jayaprakasha GK, Jaganmohan R, Phenolic constituents from lichen *Parmotrema stuppeum* (Nyl.) Hale and their antioxidant activity. Zeitschrift für Naturforschung., 55: 1018-1022 (2000).
15. Jayaprakasha GK, Selvi T, Sakariah KK, Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. Food. Res. Int., 36: 117-122 (2003).
16. Jayaprakasha GK, Singh RP, Sakariah KK, Antioxidant activity of grape seed extracts on peroxidation models. J.Agric. Food. Chem., 55:1018-1022 (2001)
17. Jomova K, Valko M, Advances in metal-induced oxidative stress and human disease**.** Toxicol., 283: 65- 87(2011).
18. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS . Antioxidant activity of plant extracts containing phenolic compounds. J. Agric. Food. Chem. 47:3954–3962(1999).
19. Kwang YA, Winne CMS, Hock EK, Hip SY, Antioxidant potential of Carica papaya peel and seed. Focus. Modern. Food. Indus., 1: 11-15(2012).
20. Lin CC, Liang, JH, Effect of antioxidants on the oxidative stability of chicken breast meat in a dispersion system. J. Food. Sci., 67(2): 530-533(2002).

21. Maisarah AM, Nurul-Amira B, Asmah R, Fauziah O, Antioxidant analysis of different parts of *Carica papaya.* Inter. Food. Res. J., 20(3): 1043-1048(2013).
22. Marinova D, Ribarova F, Atanasova M, Total phenolics and flavonoids in Bulgarian fruits and vegetables. J. Univ. Chem. Tech. Metall., 40: 255-260(2005).
23. Meyer A, Heinomen M, Frankel E, Antioxidant interactions of catechin, cyanidin, caffeic acid, quercetin and ellagic acid on human LDL oxidation. Food.Chem., 61: 71–75. (1998)
24. Mituda H, Yasumoto K, Iwami K, Antioxidative action of indole compounds during auto oxidation of linoleic acid. Eiyoto. Shokuryo., 19: 210-214(1966).
25. Norshazila S, Zahir S, Mustapha S, Aisyah MR, Rahim K, Antioxidant levels and activities of selected seeds of Malaysian tropical fruits. Mal. J. Nutr., 16(1): 149-159 (2010).
26. Osawa T, Namiki M, Natural antioxidants isolated from eucalyptus leaf waxes. J. Agric. Food Chem. 33: 777-780 (1985).
27. Oyaizu M, Studies on product browning reaction: antioxidant activity of products of browning reaction prepared from glucosamine. J. Nutri. 44: 307-315(1986).
28. Prasad KN, Chew LY, Khoo HE, Yang B, Azlan A, Ismail A, Carotenoids and antioxidant capacities from *Canarium odontophyllum* Miq. fruit. Food Chem., 124: 1549-1555(2010).
29. Ramchandani AG, Chettiyar RS, and Pakhale SS, Evaluation of antioxidant and anti-initiating activities of crude polyphenolic extracts from seedless and seeded Indian grapes. Food. Chem., 119(1):298–305(2010).
30. Saito M, Hosoyama H, Ariga T, Kataoka S, Yamaji N, Anti ulcer activity of grape seed extract and procyanidins. J. Agric. Food. Chem., 46:1460-1464(1998).
31. Serra AT, Matias AA, Nunes AVM, Leitao MC, Brito D, Bronze R, *In vitro* evaluation of olive and grape based natural extracts as potential preservatives in food. Inno.Food Sci. and emer. technol. 9: 311-319(2008).
32. Shahidi F, Wanasundara PKJPD, Phenolic antioxidants. Cri. Rev. Food. Sci. Nutr., 32:67-103(1992).
33. Tagurt T, Tanka T, Kouno I, Antimicrobial activity of 10 different plant polyphenols against bacteria causing food borne diseases. Biol. Pharm. Bull., 27:1965-1969(2004).
34. Thiago ASA, Nelson LA, Elba LCA, and Ulysses PA, A new approach to study medicinal plants with tannins and flavanoids contents from the local knowledge.J. Ethnopharmacol., 120: 72-80 (2008).
35. Yen G, Wu J, Antioxidant and radical scavenging properties of extracts from Ganoderma tsugae. Food. chem., 65: 375-379(1999).
36. Yi OS, Meyer AS, Frankel EN. Effect of phenolic grape extracts on the oxidation of lecithin liposomes. J. Am. Oil. Chem. Soc. 74: 1301-1307(1997).
37. Zhang A, Fang Y, Wang H, Li H, Zhang Z, Free-Radical Scavenging Properties and Reducing Power of Grape Cane Extracts from 11 Selected Grape Cultivars Widely Grown in China. Molecules. 16: 10104-10122 (2011).
38. Zhou K, Wang H, Mei W. Li X, Luo Y, Dai H, Antioxidant Activity of Papaya Seed Extracts. Molecules. 16:6179-6192(2011).