Antioxidant capacity and free radical scavenging activity of *Pterocarpus marsupium* and *Vitis vinifera* by using different *in vitro* model-A comparative study

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ABSTRACT:

The present study was to evaluate the antioxidant potential and the total phenolic content of *P.marsupium* bark and *V.vinifera* seeds extract. Antioxidant potential of selected plants was investigated in aqueous and ethanolic solvent under two conditions 1hr and overnight extraction. The free radical scavenging activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay in the concentration range of 0.5 mg/ml-30.0 mg/ml .The scavenging activity of all the extracts was compared with standards namely Butylated hydroxyl toluene (BHT), Gallic acid and Ascorbic acid. IC_{50} of aqueous *P. marsupium* overnight extract was found to be 1.10 ± 0.263 mg/ml and that of aqueous *V.vinifera* (1hr) extract was found to be 3.075 ± 0.074 mg/ml for DPPH. Total phenolic content was determined by Folin-Ciocalteau method and expressed in terms of Gallic acid equivalent .The aqueous overnight extract of *P. marsupium* and ethanolic overnight extract of *V.vinifera* showed highest phenolic content (30.88 ± 0.090 mg/ml and 33 ± 0.115 mg/ml) respectively. *P. marsupium* bark and *V. vinifera* seeds extract showed significant antioxidant activity as compared to standards ($p < 0.05$). The ferric reducing capacity was measured using ferrozine assay. The reducing power of the extracts increased with increasing concentration.

Keywords: Antioxidant, DPPH, Total phenolic content, ferrozine

1. INTRODUCTION

Medicinal plants have played a pivotal role in the health care of ancient and modern culture. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various human ailments as they are a good source of therapeutic agents and also non-toxic as compared to modern medicines. Plant based antioxidants especially the phenolics have gained considerable importance due to potential health benefits (Narayanaswamy Nithya *et al*., 2011).

Antioxidants are very important as they protect the human body against oxidative damage caused by free radicals. An antioxidant is a molecule which is capable of terminating oxidative chain reactions by removing free radical intermediates and inhibits other molecular oxidation. Oxidative free radicals are highly reactive to attack molecules by capturing electrons and thus modifying chemical structures. The free radicals are generated through the oxidation of carbohydrates, fats, and proteins through both aerobic and anaerobic processes. Formation of free radical is a continuous process inside our body and overproduction of the free radicals leads to oxidative stress (Dontha Sunitha, 2016). In the human body there are various enzyme systems for free radical scavenging, but micronutrients such as vitamin E, Beta-carotene and vitamin C are major antioxidants. These must be provided in diet like fruits and vegetables as body cannot produce these nutrients (Mandic I. Anamarija *et al.*, 2009).Research on antioxidants and medicinal plants has gained enormous popularity and has emerged as a potential therapeutic to prevent free radical generated damage in the human body.

Pterocarpus marsupium (Vijaysar) is a well-known plant with its Indian and ayurvedic roots. *P.marsupium* belongs to a group of '*Rasayana'* plants, which is common for its antioxidant properties. Studies have shown *P.marsupium* to be a good source of polyphenols. The extract of *P.marsupium* also contains flavonoid fraction which is found to regenerate pancreatic beta-cells (Cao Chuanhai*et al*., 2018; Losso N. Jack*et al.*, 2007).

The bark is used as an astringent and in the treatment of inflammation, stomach ache, cholera and toothache. Juice of the bark is used in the treatment of jaundice. The aqueous infusion of the bark is said to be of use in diabetes and water stored in tumbler made from the bark is reported to have anti-diabetic qualities (Tripathy B B *et al.*, 2012*; Dhiman* Kumar Anil, 2006).

Vitis vinifera (Grapes) are among the most widely consumed fruits. The demand for grapes and its products is increasing because of the associated health benefits. Grapes are rich in phenolic compounds with approximately 75% of polyphenols in grape seeds. Many scientists reported that grape seed possess free radical scavenging activity. Studies have shown that resveratrol is one of the strongest known natural antioxidant which is found in large quantities in black grape seeds (Meral Raciye *et al.*, 2017)

Grape seeds may be used to treat a range of health problems related to radical damage including heart disease, diabetes and cancer. Also it may help with a type of poor circulation and high cholesterol. It also reduces swelling caused by injury and helps in eye disease related to diabetes. There are several studies reported that grape seed may be alternative to synthetic antioxidants (Ghafoor Kashif *et al*., 2009).

2. MATERIALS AND METHODS

2.1 Chemicals

The chemicals and the reagents, DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, Folin-ciocalteau reagent, Na_2CO_3,Ferrozine , Ferric ammonium sulphate ($NH_4FeSO_4.12H_2O$), Gallic acid, tertbutyl-4-hydroxy toluene (BHT) and ascorbic acid (Vitamin C) were procured from S.D fine chemicals. All the solvents and reagents were of analytical grade.

2.2 Preparation of DPPH

DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution was prepared by dissolving 48mg of DPPH in 250 ml of ethanol.

2.3 Preparation of ferric-ferrozine

a) 0.024g of $NH_4Fe(SO_4)_2.12H_2O$ was dissolved in small amount of water.
b) 0.123g of ferrozine was dissolved in minimum amount of water followed by 1ml of 1M HCl.

a) and b) were mixed and diluted to 25 ml in standard flask with distilled water to make final iron(III) concentration($2.0x10^{-3}$M) and ferrozine concentration($1.0x10^{-2}$M).This is ferric-ferrozine complex solution.

c) Buffer solution of pH 5.5 was prepared by mixing 0.2M CH_3COOH (50ml) and 0.2M CH_3COONa (50ml)

2.4 Collection of sample

The plant samples were collected from a local market in Mumbai. For the analysis purpose, the bark of *Pterocarpus marsupium* and seeds of *Vitis vinifera* were used. After the collection, it was shade dried. The plant samples were separately pulverized to obtain a fine powder. This powder was sieved and then stored in an air tight container.

2.5 Preparation of extracts

The ethanolic extract (Et) and aqueous extract (Aq) of the plants was prepared by taking 500 mg in 10ml of the solvent .The extraction was carried out under two different conditions viz. keeping the sample on a shaker for an hour and for 24 hrs. at room temperature. It was then filtered through Whatman filter paper no.41 and the volume was made up to 25ml with appropriate solvent.

2.6 Antioxidant assays

2.6.1 DPPH assay (Free radical scavenging capacity)

The free radical scavenging activity of different extracts was determined by using DPPH assay. The decrease in the absorption of the DPPH solution after the addition of an antioxidant was measured at 530 nm. Ascorbic acid (100mg/25ml water), Gallic acid (100mg/25ml ethanol), Butylated hydroxy toluene (BHT) (100mg/25ml ethanol) were used as standards. Different concentrations (0.5-30mg/ml) of the plant extracts, 2 ml of DPPH reagent were added and the final volume was made up to 4 ml with distilled water. The tubes containing reaction mixture were incubated at room temperature for 30 minutes. After 30 minutes, the absorbance of the mixture was measured at 530 nm. Distilled water was used as a blank.

The percentage scavenging activity (%) at different concentrations was determined and the IC_{50} values of the fractions were compared with that of the standards.
Scavenging activity was calculated by using the following formula (Mehrdad Abootalebian *et al.*, 2016).

$$\%\text{Scavenging activity} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

2.6.2 Determination of total phenolic content

The total phenolic content was determined spectrophotometrically according to the Folin-Ciocalteau method and Gallic acid was used as a standard. Different aliquots of Gallic acid (0.025-2 ml) were added to the test tubes containing 1.0 ml of Folin-Ciocalteau reagent. After 3 minutes, aqueous sodium carbonate (10ml, 1M) was added to each tube and the volume was made up to 16 ml. The tubes were mixed thoroughly and allowed to stand at room temperature for 30 minutes. Absorbance of the resultant solution was measured at 550 nm. The total phenolic content was expressed in mg as Gallic acid equivalents per gram of dried plant powder (mg GAE/g) (Berker Isil Kadriye *et al.*, 2010; Narayanaswamy Nithya *et al.*, 2011).

2.6.3 Ferrozine assay (Ferric reducing assay)

The reducing power was measured by Ferrozine assay. The extracts at various concentrations (0.5-20 mg/ml) was mixed with ferric-ferrozine solution (1.5 ml), followed by the addition of buffer solution (2.0 ml, pH 5.5) and the total volume was made up to 4.5 ml using distilled water. The tubes were shaken and allowed to stand for 30 minutes at room temperature and absorbance was measured at 562 nm (Singelton VL *et al.*, 1965).

2.7 Statistical analysis

The direction and magnitude of correlation between the variables was done using students t- test. The p-value less than 0.05 were considered to be statistically significant.

3. RESULT AND DICUSSION

3.1 DPPH ASSAY

The discoloration of the DPPH reagent indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability. The different extracts of the plant showed significant scavenging activity ($p<0.05$).The scavenging activity was found to increase with increase in concentration of the plant. The aqueous overnight extract of *P.marsupium* showed highest inhibition of 65.60% (Fig. 1) and IC_{50} = 1.10 ±0.263 mg/ml (Table 1) and the aqueous overnight extract of *V.vinifera* showed highest inhibition 39.3% (Fig.2) and IC_{50} = 6.0 ±0.065 mg/ml (Table 1) . The radical scavenging activity of aqueous overnight extract of *P.marsupium* was found to be significantly higher than aqueous overnight extract of *V.vinifera* (Fig.3).

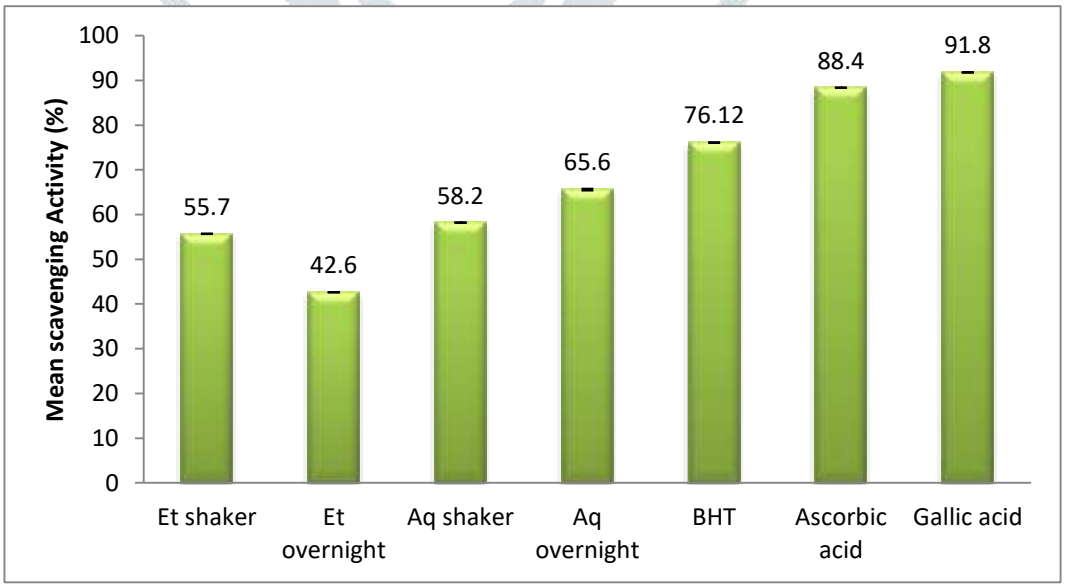

Fig 1: DPPH Radical Scavenging Activity of *P. marsupium*.

The data are expressed as mean value ±SD (n=3).All values are significant $p<0.05$

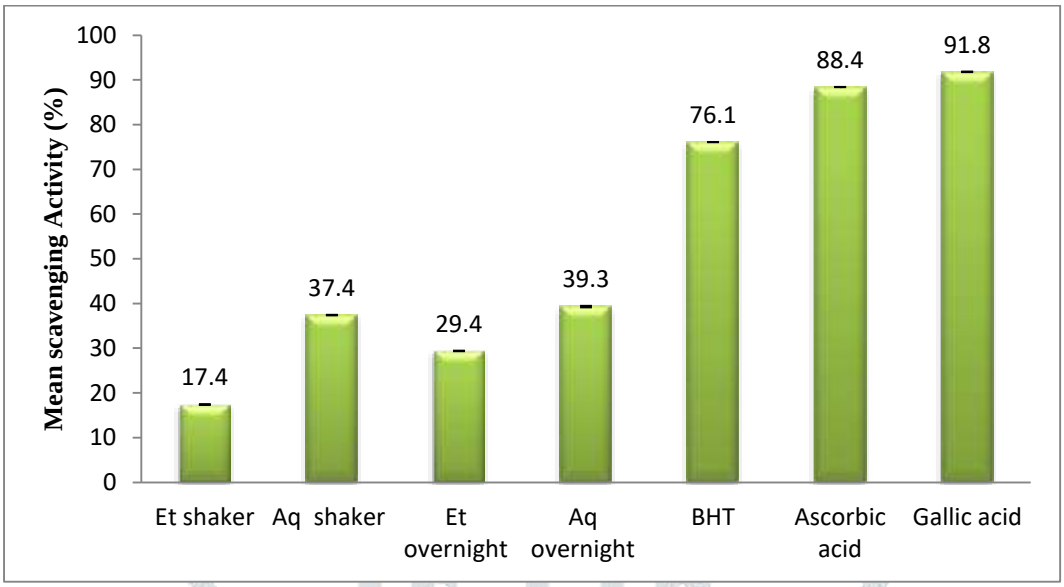

Fig 2: DPPH Radical Scavenging Activity of *V. vinifera*

The data are expressed as mean value ±SD (n=3).All values are significant $p<0.05$

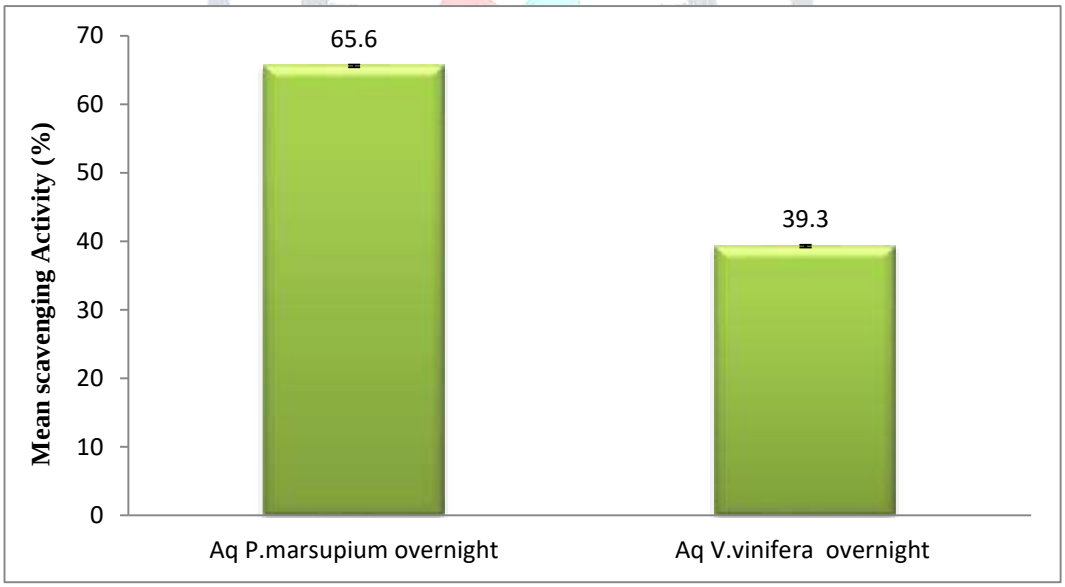

Fig.3: Comparison of Mean Scavenging Activity (%) of *P. marsupium* and *V. vinifera*

All values are significant $p<0.05$

TABLE 1: IC_{50} values of *P. marsupium* and *V. vinifera*

SAMPLES	DPPH IC_{50} values (mg/ml)
Ethanolic *P.marsupium* (Overnight)	2.675 ±0.87
Aqueous *P.marsupium* (Overnight)	1.10 ±0.263
Ethanolic *P.marsupium* (1hr)	1.825 ±0.67
Aqueous *P.marsupium* (1hr)	1.725 ±0.124
Ethanolic *V.vinifera* (Overnight)	3.875 ±0.098
Aqueous *V.vinifera* (Overnight)	6.0 ±0.065
Ethanolic *V.vinifera* (1hr)	4.125 ±0.182
Aqueous *V.vinifera* (1hr)	3.075 ±0.074
Gallic acid	0.46 ±0.116
Ascorbic acid	0.54 ±0.454

3.2 TOTAL PHENOLIC CONTENT-FOLIN-CIOCALTEAU METHOD.

The aqueous extract (overnight) of *P.marsupium* showed highest phenolic content of 30.88 ± 0.090 mg GAE/g and ethanolic extract (overnight) *V.vinifera* showed highest phenolic content of 33 ± 0.115mg GAE/g of dried plant powder. The total phenolic content were expressed as regression equation of the calibration curve. (Fig 4)

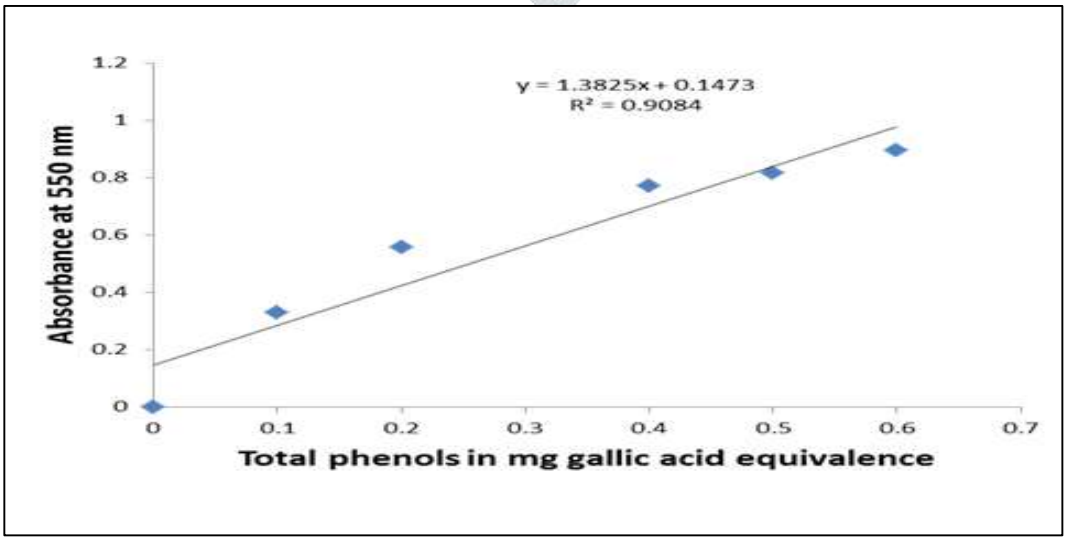

Fig 4: Total phenolic assay

Table no. 2: Total phenolic content of *Pterocarpus marsupium* and *Vitis vinifera*

SAMPLE	TOTAL PHENOLIC CONTENT (mg GAE/g)
Ethanolic *P.marsupium* (Overnight)	24.32 ±0.236
Aqueous *P.marsupium* (Overnight)	30.88 ±0.090
Ethanolic *P.marsupium* (1hr)	15.04 ±0.152
Aqueous *P.marsupium* (1hr)	15.92 ±0.194
Ethanolic *V.vinifera* (Overnight)	33 ±0.115
Aqueous *V.vinifera* (Overnight)	4.28 ±0.094
Ethanolic *V.vinifera* (1hr)	31.36 ±0.257
Aqueous *V.vinifera* (1hr)	2.76 ±0.046

Data are presented as mean ±standard deviation

3.3 FERROZINE ASSAY

Reducing power was measured by direct electron donation which leads to the reduction of Fe^{+3} to Fe^{+2}. The product was visualized by the formation of an intense magenta colour complex. The higher the absorbance value, stronger is the reducing power of the samples. The reducing power of *V.vinifera* was found to increase linearly with the concentration ($R^2 = 0.9413$). The reducing power of *P.marsupium* showed linear response till 4mg/ml and then it remained constant.

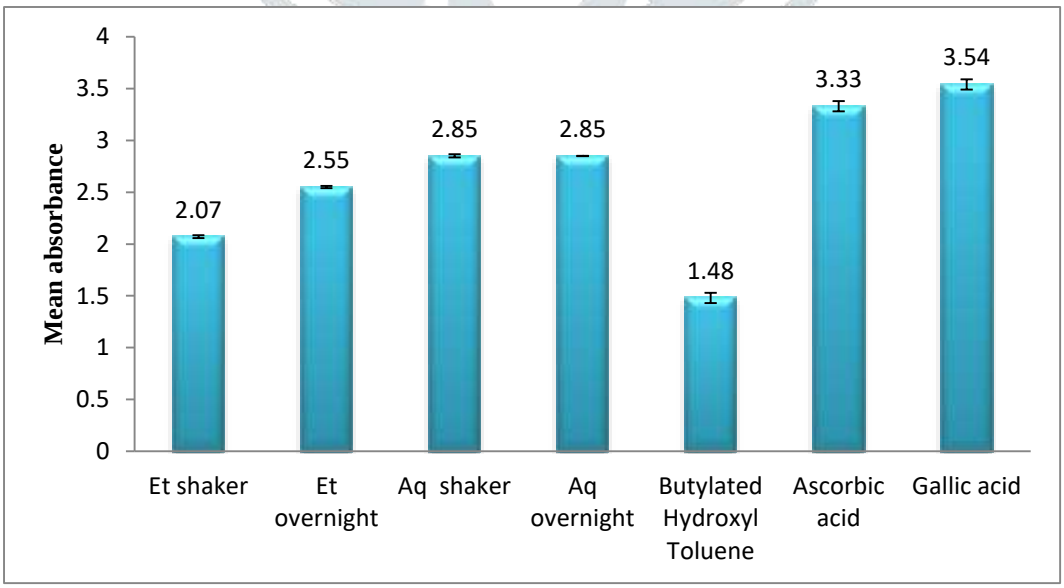

Fig 5: Ferric reducing capacity of *P.marsupium*

The data are expressed as mean value ±SD (n=3).All values are significant $p<0.05$

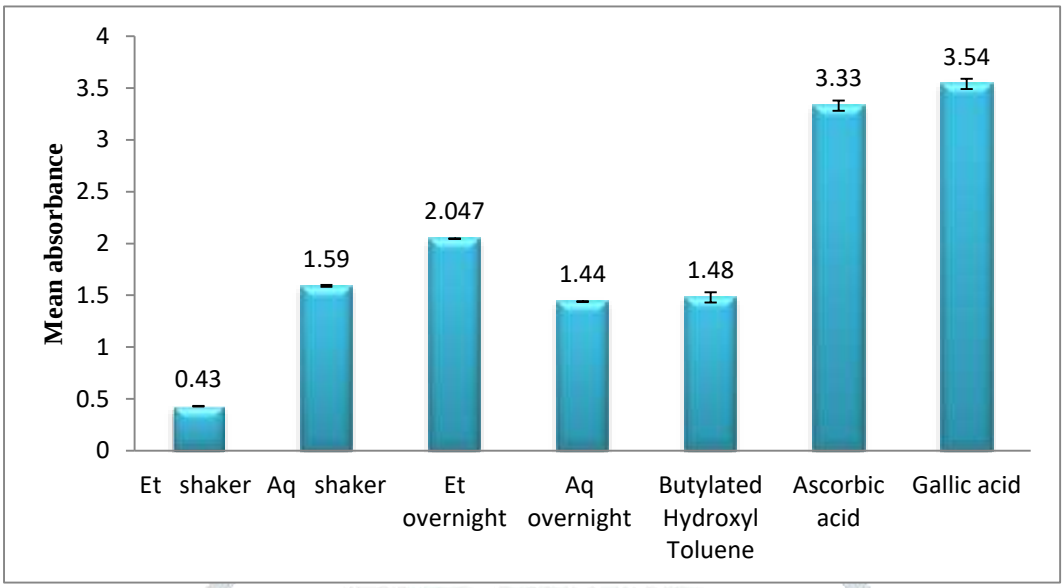

Fig 6: Ferric reducing capacity of *V.vinifera*
The data are expressed as mean value ±SD (n=3).All values are significant p<0.05

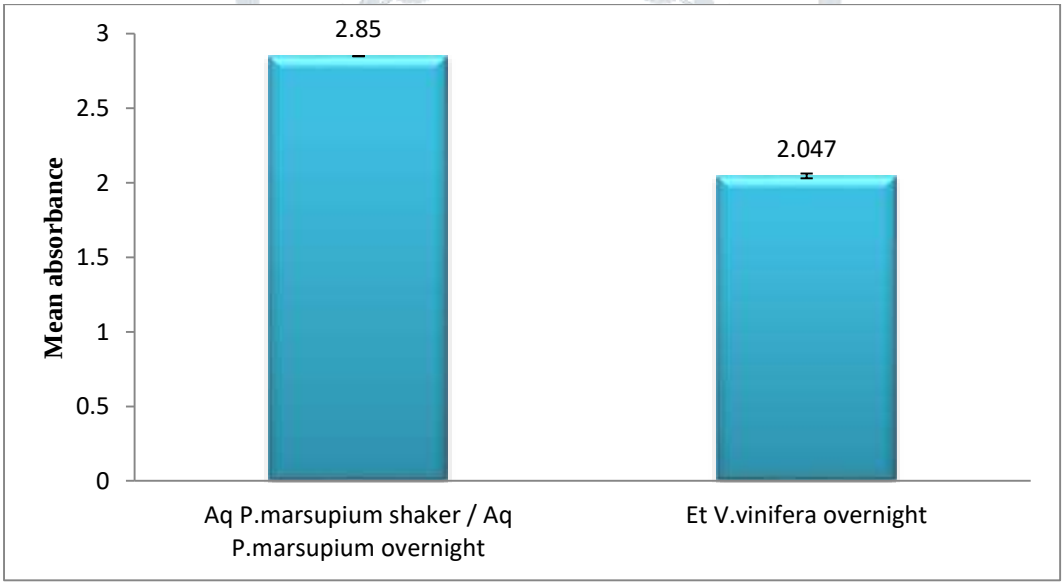

Fig.7 Comparison of mean absorbance of *P. marsupium* and *V. vinifera*
All values are significant p<0.05

CONCLUSION

The results indicate selected plants to be a potential antioxidant agent. In this study, we conclude that *P.marsupium* and *V.vinifera* showed antioxidant property and this potency could be attributed to the presence of phenolic compounds in *P.marsupium* but no such correlation was observed in *V.vinifera*, so the radical scavenging activity of *V.vinifera* could be due to other phytoconstituents. *P.marsupium* is on the verge of extinction but *V.vinifera* seed is a waste from winery, an easily accessible source.

The phytochemicals responsible for the antioxidative activity of *V.vinifera* and *P.marsupium* are currently unclear. So there is scope to explore in the area of isolation and identification of the phytochemicals constituents of these plants.

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REFERENCES

1. Narayanaswamy Nithya, Balakrishnan K P, Evaluation of some Medicinal plants for their antioxidant properties, International Journal of PharmTech Research, 2011, Vol. 3, No.1, pp 381-385.
2. DonthaSunitha, A review on antioxidant methods, Asian journal of pharmaceutical and clinical research, Vol 9, suppl.2, 2016.
3. Mandic I. Anamarija, Djilas M. Sonja, Canadanovic- Brunet M. Jasna, Cetkovic S. Gordana, Vulic J. Jelena, Amntioxidant activity of white grape seed extracts on DPPH Radicals, (2009)40, 53-61.
4. Cao Chuanhai, PathakSaravadhan, PatilKiran, Antioxidant Nutraceuticals: Preventive and Health care applications, Chp 12.3.1.1,2018.
5. Losso N. Jack, ShahideFereidoon, Anti- angiogenic functional and medical foods, 2007.
6. Tripathy B B, Chandalia B. Hemraj, Dar Kumar Ashok, RSSDI Textbook of Diabetes Mellitus, 2012.
7. Dhiman Kumar Anil, Ayurvedic drug Plants, 2006.
8. MeralRaciye, ErturkBurcu, Antioxidant Activity and phenolic profiles of grape seed, International Congress on Medicinal and aromatic plants, 2017, 10-12.
9. GhafoorKashif, Choi HeeYong,Optimization of Ultra sound assisted extraction of phenolic compounds and antioxidants from grape peel through response surface methodology, 2009, 295-300.
10. MehrdadAbootalebian, JavadKeramat, Mahdi Kadivar, FarhadAhmadi, MahnazAbdinian,Comparison of total phenolic and antioxidant activity of diffetrent*Menthaspicata*and*M.longifolia* accessions,volume61,issue2, December 2016,pg:175-179
11. BerkerIsilKadriye, Guclu, Kubilay, DermirataBirsenApakResat, A novel antioxidant assay of ferric reducing capacity measurement using ferrozine as the colour forming complexation reagent,1770-1778, 2010.
12. Singelton VL, Joseph AR, Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents, Am. J. Enol. Votic, 16:3, 1965, 144-158.