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RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY OF *VITIS VINIFERA, CAMELLIA SINENSIS AND ZINGIBER OFFICINALE* EXTRACTS AND THEIR COMBINATIONS

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ABSTRACT

The present study is undertaken to find out any synergestic effect of mixture of the selected plant extracts in supplying polyphenolic compounds which can be useful as an antioxidants. In the present study, antioxidant activity, reductant activity and content of total flavanoids are estimated in individual selected plant extracts, combination of mixture of two selected plant extracts in the proportion of 1:1 and mixture of all the three plant extracts in the proportion of 1:1:1. The antioxidant activity is measured by β- carotene linoleate model system, reductant activity is measured by the method of Yen and Duh and total flavanols are measured by vanillin method using catechin as a standard. In the present study we observed highest level of antioxidant activity and reducing power are found in a mixture of Vitis vinifera, Camellia sinensis L. and Zinziber officinale in the proportion of 1:1:1

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INTRODUCTION

Food products, medicines or cosmetic products continuously undergoes oxidation during their process, storage and application which may responsible for quality deterioration of products, affects human body by triggering a number of diseases due to lipid peroxidation and increases of aging. Food industries, pharmaceuticals and cosmetic industries use various antioxidants in their product. These agents capture the free radicals and bind them in low reactive, stable systems. The most commonly used antioxidants in industries are vitamin C, vitamin E, Q10 coenzymes, gallic acid, ferrulic acid, lipoic acid, butylated hydroxyl toluene (BHT), butylated hydroxyl anisole (BHA). The synthetic antioxidants are reported to be carcinogenic (Madavi, 1995). Therefore the search of natural antioxidants, especially of plant origin, has greately increased in recent years (Loliger, 1991). Grape (*Vitis vinifera)* is one of the largest growing crop, especially in and around western Maharashtra. Various phenolic compounds have been reported in grapes.Grape seeds are rich source of monomeric phenolic compounds, such as (+) catechin, (-) epicatechin and (-) epicatechin-o-gallate and dimeric, trimeric, tetrameric procyanidines (Fuleki, 1997).

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These compounds act as antioxidants (Jayaprakasha, 2001), antimutagenic and antiviral agents (Saito *et al.,* 1998). Phenolics in grape seeds have been reported to inhibit low density lipoproteins in humans and oxidation *in vitro* (Frankel *et al.,* 1995). Studies have reported possible use of phenolics in grapes in preventing atherosclerosis (Kovac, 1991). By considering these health benefits the grape seeds have been used as dietary suppliments. Green tea (*Camellia sinensis L.)* is cultivated in about 30 countries worldwide. In India it is mostly grown in Kerala, Assam and in parts of West Bengal. Tea (*Camellia sinensis L.)* contains large amount of polyphenolic compounds with antioxidant properties and may prevent oxidative damage of DNA (Wiseman, 2008; Buschman, 1998). Due to large content of flavanoids and other polyphenols, green tea shows anticarcinogenic, antioxidative and hypolipidaemic properties (Buschman, 1998; Yang, 1999), anti-inflammatory (Tipoe *et al.,* 2007), cholesterol lowering (Koo, 2007), antiviral and antibacterial properties (15) and antioxidant (Hajimahamoodi *et al.,* 2008). By considering these properties, the green tea can be used as dietary suppliment and ingradient in cosmetics, shampoos, sweet waters and antiageing emulsions (Pykowska, 2002; Wang *et al.,* 2002; Frankel, 1997; Ahmed, 1999; Elzbieta, 2011). Ginger (*Zingiber officinale)* is widely cultivated plant worldwide. It is used as carminative, diaphoric, antispasmodic, expectorant, circulatory stimulant, astringent, appetite stimulant, diuretic, digestive and anti-inflammatory agent (Van

Wyk, 2013; Jiang *et al.,* 2006). The dried rhizomes of the herb are used traditionally to cure human ailments. It is used to cure diarrhea, dysentery, fever, cough, ulcer, boils and wounds (Young *et al.,* 2005), protective effect against alcohol induced renal damage (Shanmugam *et al.,* 2010).

MATERIALS AND METHODS

Chemicals- All chemicals used in the present study were Analar grade (AR), and obtained from Merk (Mumbai, India), Ranbaxy (New Dehli, India) and HiMedia (Mumbai, India)

Preparation of Grape Seed (*Vitis vinifera)* extract

The grape seeds were procured from Sula vineyards, Nasik. The seeds were dried in shade. They were griend and powdered mechanically with the help of porcelain mortor and pestle. Exactly 100 gm. of powder was extracted in soxhlet extractor with hexane for 6 hours for removal of fatty material. The defated seed powder was extracted in soxhlet extractor for 5 hours with 300 ml. mixture of ethyl acetate: water, having the ratio of 17:3. The extract was pooled, dried with anhydrous sodium sulphate and concentrated under vacuum to yield a viscous liquid. Procyanidins were precipitated by adding double volme of hexane to viscous liquid. The precipitate was collected by filtration under vacuum. The extract obtained was weighed and stored in dessicator.

Preparation of Green tea (*Camellia sinensis L.)* extract

100 gm. packaged long leaf green tea was purchased from local super market of Tately brand with 12 month expiry date of batch code- 24TT118. 50 gm. of green tea leaves were mechanically powdered in a porcelain crucible. The powder then mixed with chloroform and petroliun ether (1:1) and kept in extraction thimble for about 2 hours to remove chlorophyll and hydrophobic substances. After separating the eluent and drying the plant material, the proper extraction was done in a soxhlet extractor by mixing the powder with 95% ethanol at a constant temperature of 70 c for 5 hours. Then the extract was pooled, dried under vacuum to yield a dark brown extract, the yield was weighed and stored in a dessicator.

Preparation of Ginger (*Zingiber officinale)* extract

Fresh rhizomes of ginger were purchased from local market Loni. They were washed with water to make them free of soil and air dried at room temperature. Air dried rhizomes of the herb (1kg) were milled into fine powder mechanically with the help of porcelain crucible, then exactly 100 gram of powder was extracted with 95% ethanol for 24 hours. The extract was recovered and 300 ml. of 95% ethanol was further added to the ginger powder and the extraction was continued for 5 hours at constant temperature of 70 C. The process was repeated three times, the three extracts were pooled together, mixed, filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator. The resultant ethanolic extract was air dried, weighed and stored in a dessicator at room temp.

Determination of antioxidant activity using β- carotene linoleate model system

The antioxidant activity of grape seed extract, green tea extract, ginger extract and the combinations of grape seed+ green tea, grape seed+ ginger, green tea + ginger and the combinations of grape seed+ green tea + ginger extract(1:1:1) was estimated by the β- carotene linoleate model system (25)
0.2 mg of β- carotene, 20 mg of linoleic acid and 200 mg of Tween-40 (Polyoxyethylene sorbitate monopalmitate) were mixed in 0.5 ml of chloroform. Chloroform was removed at 40 C in a constant temperature water bath. The resulting mixture was immediately diluted with 10 ml of double distilled water and was mixed well for 1-2 min. The emulsion was further made upto 50 ml. with double distilled water. Aliquots (4 ml) of this emulsion were transferred into different test tubes containing 0.2 ml of test samples of varying concentration in methanol : water mixture (6:4 v/v). Butylated Hydroxy Toluene (BHT) was used for comparative purpose as a standard. A control, containing 0.2 ml ethanol and 4 ml of the above emulsion was prepared. All the test tubes were placed at 50 C in a constant temperature water bath. Absorbances of all the samples at 470 nm were taken at zero time (t= 0) on Helios spectrophotometer. Measurement of absorbance was continued until the colour of β- carotene disappeared in the control reaction (t= 180 min) at 15 min. intervals.A mixture prepared as above without β- carotene served as blank.All the determinations were carried out in triplicate. Dose response relationships of antioxidant activity for grape seed extract, green tea extract, ginger extract and combination of grape seed + green tea extracts (1:1), grape seed + ginger extracts (1:1) , green tea + ginger extracts (1:1) and a mixture of grape seed+ green tea + ginger (1:1:1) extracts were determined at different concentrations. The antioxidant activity (AA) of the extracts was evaluated in terms of bleaching of β- carotene using the following formula (25).

$$AA = (1- (A0-A1/A0'/A1')$$

Where A0 and A0' are the absorbance values measured at zero time of the incubation for test sample and control. A1 and A1' are the absorbance measured in the test sample and control, resp. after incubation time.

Determination of reducing power

The reducing power of the test samples were determined by the method of Yen and Duh (1993) (Yen, 1993). Different concentrations of all the three extracts and their combination of extracts (100, 200,300,400 and 500 ug/ ml) in 1 ml. methanol were mixed with 2.5 ml. of phosphate buffer (0.2 M, PH 6.6) and 2.5 ml.1 % potassium ferricyanide in 10 ml. centrifuge tubes. The mixtures were incubated for 20 min. at 50 C. At the end of the incubation, 2.5 ml. of 10% trichloro acetic acid was added to the mixtures, followed by centrifuging at 5000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml. of distilled water and 0.5 ml. of 0.1 % ferric chloride and the absorbance was measured at 700 nm. On Helios spectrophotometer. All the tests were done in triplicate. Increase in absorbance of the reaction mixture indicated the reducing power of the sample. The graph of concentration of different plant extracts against absorbance are plotted.

Estimation of Total Flavanols

The amount of total flavanols was estimated colorimetrically by the vanillin method using catechin as a standard (Price, 1978). 5.0 ml of 0.5 % vanillin in methanol was added to 1.0 ml. of methanolic grape seed extract, green tea extract, ginger extract, combination of grape seed + green tea extract, grapeseed + ginger extract, green tea + ginger extract and

mixture of grape seed+ green tea + ginger extract. The absorbance of sample and blank were measured at 500 nm. After 20 minutes in dark at room temperature. The absorbance of blank was subtracted from the absorbance of sample. The content of total flavanols in various extracts was expressed as catechin equivalents per 100 gram of extract. The tests were done in triplicate and the average results were tabulated. Statistical analysis –Statistically One Way Analysis Of Variance (ANOVA) is applied to compare the antioxidant activity between individual plant extract and combination of selected different plant extracts. If the p value is < 0.0001, it considered extremely significant and Tukey- Kramer multiple comparisons test is applied to compare antioxidant activity between selected plant extracts. If the value of q is greater than 4.897, then the p value is less than 0.05.

RESULTS AND DISCUSSION

From Table 1, it is observed that, as the concentration increases, there is increased antioxidant activity is observed in individual plant extract.

is observed ($p>0.05$) at all the four concentrations. Antioxidant activity of *Vitis vinifera* when compared against a mixture of *Camellia sinensis and Zinziber officinale,*at 1:1 proportion,significantly decreased antioxidant activity is observed in *Vitis vinifera. ($p<0.001$)at* 50 µg/ml, 75 µg/ml and 100 µg/ml. Highly significant decreased antioxidant activity is observed in *Vitis vinifera compared against a mixture of Vitis vinifera, Camellia sinensis and Zinziber officinale,*at 1:1:1 proportion ($p<0.001$) Highly significant decreased antioxidant activity is observed in *Vitis vinifera compared against BHT* at all the four concentrations ($p<0.001$). Antioxidant activity of *Camellia sinensis* when compared against *Zinziber officinale,* it is found to be significantly increases in all the four concentrations ($p<0.001$). When antioxidant activity of *Camellia sinensis is compared against a mixture of Vitis vinifera and Camellia sinensis, mixture of Vitis vinifera and Zinziber officinale,* at a proportion of1:1 it is significantly decreases ($p<0.001$) in all the four concentrations. and also significantly decreases when compared against mixture of all the three selected plant extracts at a proportion of 1:1:1 ($p<0.001$).

Table 1.

Extracts	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Grape seed extract (*Vitis vinifera)*	32.4± 0.64	53.6±0.75	72.87±1.31	90.37±1.31
Green tea extract (*Camellia sinensis)*	30.5±1.05	52.9±0.65	70.17±1.35	89.67±1.31
Ginger extract *(Zinziber officinale)*	27.16±0.713	46.4±0.45	66.83±1.19	79.77±1.16
Grape seed + green tea extract	35.83±0.286	62±0.51	84.07±0.74	94.97±1.25
Grape seed + ginger extract	32.23±0.953	55.17±0.69	74.43±0.82	92.83±1.35
Green tea + ginger extract	35.1±0.941	60.03±0.88	77.43±1.14	91.9±1.35
Grape seed +green tea + ginger extract	35.77±0.78	65.8±0.55	87.8±1.07	96.03±0.78
BHT standard	37.93±1.20	67.33±1.13	89.77±0.71	97.23±0.71

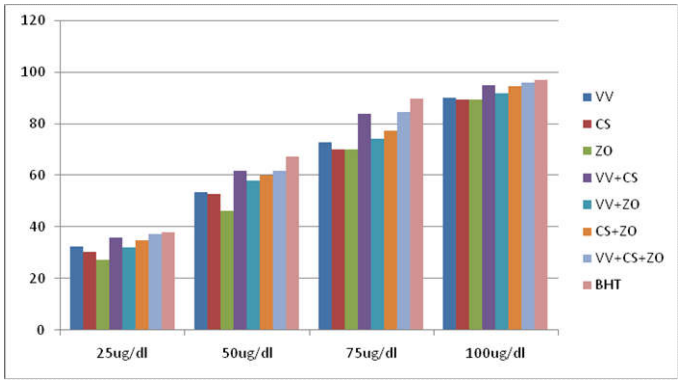

Figure 1. Antioxidant activity of selected plant extracts

The values obtained by grape seed extract correlates with the study of G. K. Jayaprakash et al. (2001). At a concentration of 25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml of *Vitis vinifera and Camellia sinensis,* there is no significant difference of antioxidant activity is found ($p>0.05$).When antioxidant activity of *Vitis vinifera* is compared against *Zinziber officinale,* there is highly significant increased level is found in *Vitis vinifera* at a concentration of 25 µg/ml and 50 µg/ml ($p<0.001$). but when the concentration increases to 75 µg/ml and 100 µg/ml, there is no significant change in antioxidant is observed in between these two plant extracts. When antioxidant activity of *Vitis vinifera* is compared against a mixture of *Vitis vinifera and Camellia sinensis* at a proportion of 1:1, there is highly significant decrease antioxidant activity is noted in *Vitis vinifera* at all the four concentrations ($p<0.001$). When antioxidant activity of *Vitis vinifera* is compared against a mixture of *Vitis vinifera and Zinziber officinale* at a proportion of 1:1, there is no significant change

Highly significant decreased antioxidant activity is observed in *Camellia sinensis* compared against *BHT* at all the four concentrations ($p<0.001$). Highly significant decreased antioxidant activity is observed in *Zinziber officinale* when compared against *Vitis vinifera and Camellia sinensis, mixture of Vitis vinifera and Zinziber officinale ,and a mixture of Camellia sinensis and Zinziber officinale* at a proportion of1:1, a mixture of *Vitis vinifera, Camellia sinensis and Zinziber officinale* at a proportion of1:1:1($p<0.001$) and against BHT. Antioxidant activity of *Vitis vinifera and Camellia sinensis when compared against Vitis vinifera and Zinziber officinale* at a proportion of 1:1, highly significant increased level is found at all the four concentrations. But when compared against *Camellia sinensis and Zinziber officinale* at a proportion of 1:1, the level is found to be not significantly changes ($p>0.05$), and also not significantly changes against mixture of all the three selected plant extracts and against BHT ($p>0.05$), When

antioxidant activity of *Vitis vinifera and Zinziber officinale* at a proportion of 1:1 compared against *Camellia sinensis and Zinziber officinale* there is not significant change is observed. ($p>0.05$) but when compared against mixture of all the three selected plant extracts at a proportion of 1:1:1 and BHT, a significant decreased level is found ($p<0.001$).

vinifera, Camellia sinensis and Zinziber officinale extracts at a proportion of 1:1:1 compared against BHT there is no significant difference is found ($p>0.05$). The mechanism involves bleaching of β – carotene, a free radical mediated phenomenon by the hydroperoxides formed from linoleic acid.

Table 2. Reducing power (µg/ml)

Extracts	100	200	300	400	500
Grape seed extract(*Vitis vinifera)*	0.45±0.02	0.59±.024	0.72±0.022	0.85±0.026	1.22±0.032
Green tea extract (*Camellia sinensis)*	0.44±0.032	0.58±0.028	0.68±0.026	0.75±0.027	1.18±0.029
Ginger extract*(Zinziber officinale)*	0.32±0.026	0.46±0.022	0.58±0.029	0.72±0.029	1.1±0.028
Grape seed + green tea extract	0.48±0.025	0.65±0.027	0.76±.030	0.92±0.022	1.28±0.025
Grape seed + ginger extract	0.46±0.22	0.62±0.028	0.73±0.019	0.88±0.27	1.25±0.028
Green tea + ginger extract	0.52±.027	0.68±0.018	0.80±0.026	0.95±0.026	1.44±0.030
Grape seed +green tea + ginger extract	0.61±0.024	0.72±0.025	0.86±0.027	0.99±.032	1.92±0.029

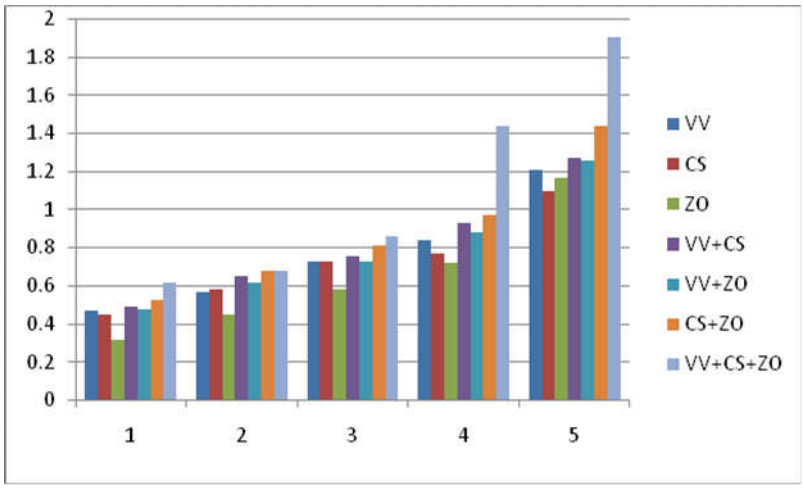

Figure 2. Reducing power of selected plant extracts

Table 3. Composition of total flavanols in different plant extracts. Values are expressed in mg. catechin / 100 gm. Extract

Component	Grape seed extract(*Vitis vinifera)*	Green tea extract (*Camellia sinensis)*	Ginger extract *(Zinziber officinale)*	Grape seed + green tea extract	Grape seed +Ginger extract	Green tea +Ginger extract	Grape seed+ Green tea + Ginger extract
Total flavanols	38.6 ±1.65	37.8±1.32	33.4± 1.28	40.1 ±1.38	39.2 ±1.33	38.3 ±1.29	43.7 ±1.22

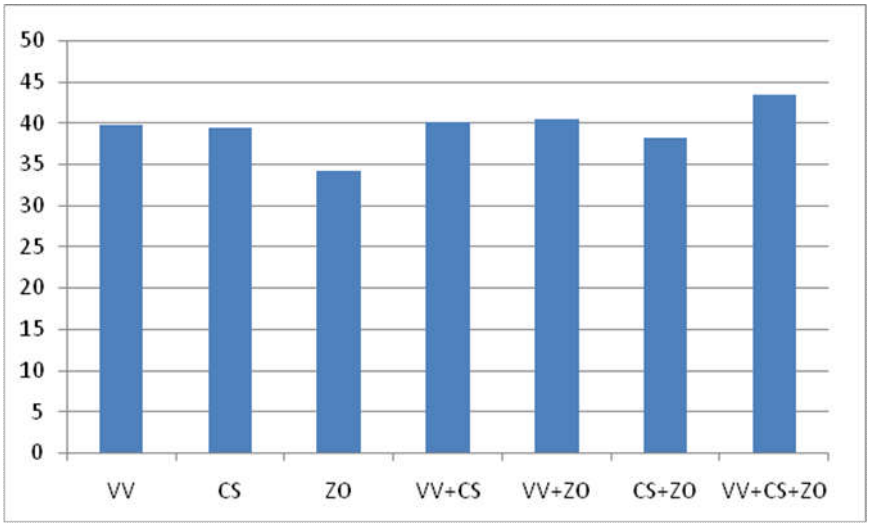

Figure 1. Total flavanols in selected plant extracts mg catechin/ 100 gm

Antioxidant activity of *Camellia sinensis and Zinziber officinale* when compared against mixture of all the three selected plant extracts at a proportion of 1:1:1 and BHT, a significant decreased level is found ($p<0.001$) But when *Vitis*

In this system, β–carotene, undergoes rapid discoloration in absence of an antioxidant. The linoleic acid free radical, formed by the abstraction of a hydrogen atom from one of its di allylic methylene groups, attacks the highly unsaturated β –

carotene molecule, as β – carotene molecule loses their double bond by oxidation, the compound loses its chromophore and characteristic orange colour, which can be monitored spectrophotometrically. The antioxidant activity of green tea leaves presented in the study correlates with M. Hajimahmoodi et.al, 2008 (Hajimahamoodi, 2008), Chan E.W.C.2007 (Chan, 2007) and Katalinic et al 2006 and antioxidant activity of ginger extract correlates with the studies done by Silvia Mosovska et al. 2015 and Shanmugam et.al, 2010 though the method was different. The antioxidant activity of selected different plant extracts is found proportionately with increasing reducing power of these plant extracts (fig 1). The reducing properties are associated with the presence of reductones (Pinn- Der, Duh, 1998). The antioxidant action is based on the breaking of free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones also react with certain precursors of peroxides, thus preventing peroxide formation. The total flavanols in the present study is found significantly more in grape seed extract as compared to other two individual plant extracts, while by combination of two plant extracts, the total flavanols are found more in grape seed + green tea extract and the highest concentration of total flavanols are found in combination of grape seed + green tea + ginger extract. The total flavanols may act in a similar way as reductions by donating electrons and reacting with free radicals to convert them to more stable products and terminating the free radical chain reaction (Jayaprakasha, 2001).

Conclusion

The results of present study shows that, the highest level of antioxidant activity, reducing power and content of total flavanols are found in the mixture of grape seed extract + green tea extract+ ginger extract at proportion of 1:1:1 as compared with any individual plant extract or a mixture of any two plant extract, and can be substituted to chemical antioxidants like BHT, which can be used to prevent the disorders arises due to lipid peroxidation and decreasing the rate of ageing. From the present study it is found that there is direct relationship between total flavanol content and reducing power, antioxidant activity in a single plant extract, combination of two plant extracts and a mixture of three plant extracts.

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