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ANTIOXIDANT ACTIVITY OF LOCALLY AVAILABLE PLANT EXTRACTS AGAINST OXIDATIVE STRESS INITIATED BY NOISE POLLUTION IN ALBINO MICE (*MUS MUSCULUS*)

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Keywords: Oxidative stress, Phytochemicals, Lipid peroxidation, Tissue homogenate

Abstract

Prolonged exposure to noise produces free radicals, which enhance oxidative stress in the body and it requires exogenous antioxidants to maintain an adequate level of antioxidants in order to balance Reactive Oxygen Species (ROS). Clove (*Syzygium aromaticum*), Kolvengi (*Nigella sativa*) leaves and Karhi Patta (*Murraya koenigii*) seeds were selected to reduce the elevated level of oxidative stress in mammals such as albino mice *(Mus musculus)*, as these are commonly used household plant spices. These plants were evaluated for their phytoconstituents and quantitative determination of these phytochemicals. Finally their antioxidant potential was examined by adopting different assay techniques. After noise exposure along with aqueous extract administration, mice livers were removed to prepare tissue homogenate for the evaluation of oxidative activity and were assessed statistically. The related plants proved their antioxidant potential to reduce noise effect by minimizing the production of free radicals.

Introduction

Sudden increase of free radicals due to environmental pollutants like stress and noise gives birth to a tragic phenomenon called oxidative stress. The oxidative stress occurs due to decreased level of antioxidant instead of propagation of ROS (Salmon *et al.* 2010). As reported 100 dB noise can cause lipid peroxidation and enhance oxidative stress (Yildirim *et al.* 2007). Hydroxyl radicals generates lipid hydro-peroxide which is unstable and forms malondialdehyde (MDA), an indicator of lipid peroxidation (Ahmed *et al.* 2013). Plants have excessive nutrients termed as phytochemicals and possess antioxidant properties (Venskutonis *et al.* 2005). Plants have the ability to repair damages caused by ROS, enhance immune defence and lower the risk of development of degenerative diseases (Pham-Huy *et al.* 2008).

Plant antioxidant behavior is due to the presence of specific phytochemicals. Antioxidant property of a plant mainly depends upon the phenolic content due to its hydroxyl group which takes part in the removal of free radical. They act as reducing and metal chelating agents. They include flavonoids, phenolic acids and tannins, all have widespread series of antioxidant accomplishments (Martins *et al.* 2011).

Materials and Methods

This study is comprised of two steps. In the first step locally grown plants commonly known as clove, Kolvengi and Karhi Patta were used. The plant material (30 g each) was crushed and their aqueous and methanol mixture (10 g :100 ml; w/v) were prepared. Plant extracts and powder were examined for the qualitative determination of phytochemicals (alkaloids, saponins, terpenoids, flavonoids, tannins, phytosterols, phenols, carotenoids, fatty acids and steroids) by standard methods (Ugochukwu *et al.* 2013, Saeed *et al.* 2012, Delphin *et al.* 2014, Obasi *e t al.*

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2010, Ajayi *et al.* 2011, Sujatha *et al.* 2013). Quantitative analysis was conducted spectrophotometerically to examine the total flavonoids content (TFC), total phenolic content (TPC), tannins content, alkaloid content and selenium determination in plants (Garg *et al.* 2012, Sreevidya and Mehrotra 2003). Scavenging of hydrogen peroxide radical, nitric oxide radical scavenging activity, total reduction capability (TRC), total antioxidant capacity was done (Behera *et al.* 2012).

In the second step 75 Swiss albino mice (*Mus musculus*) were used for determination of antioxidant potential of these plants against noise. They were fed with special mice feed and fresh drinking water. Fifteen mice in each group were randomly distributed according to treatment with plant extract (Table 1). All experimental groups were subjected to noise stimulation that ranged from 95 - 100 dB for one month (5 hrs daily). Experimental groups were given different plant extracts mixed with drinking water for that purpose 1ml of extract/bodyweight/g in 100 times dilution was used.

Table 1. Scheme showing control (C) and experimental groups (A_1, A_2 and A_3) distributed according to their administrative patterns with plant treatment.

Control group (C)	No. plant extract and noise administration
Experimental groups	
A1	100 dB noise and fresh drinking water
A2	100 dB noise and clove extract
A3	100 dB noise and kolvengi extract

During noise exposure all groups were keenly observed for their morphological abnormalities, weight and their tissue homogenate was analyzed for the level of malondialdehyde (MDA) and nitrite with the help of spectrophotometer. For comparing the differences between experimental and control groups one-way analysis of variance (ANOVA-single factor) was performed and tabulated according to their level of significance. Regression (Intercept and slope) was used to determine the diversions in scavenging activity of selected plants with respect to standard.

Results and Discussion

Current results depicted that chosen plants have shown significant antioxidant ability (Table 2). Amin *et al.* (2013) stated the presence of alkaloids, phenolic compounds and flavonoids in clove.

The aqueous and methanol extracts of plants showed significant levels of total flavonoids, phenolic content, tannins, alkaloid content and selenium analysis with slight differences (Table 3). Antioxidant potential of a plant mainly depends on its phenolic content. All selected plants in current study owned substantial phenolic content. There is a correlation between antioxidant ability of a plant and phenols (Ifesan *et al.* 2013).

Hydrogen peroxide radical scavenging activity in methanol extract, more noticeable. H_2O_2 has the tendency to produce hydroxyl radical, which causes lipid peroxidation. Clove and Karhi Patta showed very significant neutralizing aptitude with an insignificant difference from Kolvengi as shown in Fig. 1.

Nitric oxide radical scavenging ability in methanol extract was descended as ascorbic acid > Kolvengi > Karhi Patta > Clove and in aqueous extract it was in the pace of ascorbic acid >

Kolvengi > Clove > Karhi Patta as shown in Fig. 2. Reduction ability of plant extracts also indicated a dose response relationship.

Table 2. Qualitative determination of plant constituents.

Phytochemicals	Experimental plants					
	Clove		Kolvengi		Karhi Patta	
	Aqueous	Methanol	Aqueous	Methanol	Aqueous	Methanol
Alkaloids	+++	+++	++	+++	+++	+++
Saponins	+	++	++	+++	+++	+
Terpenoids	+++	+++	-	-	++	++
Flavonoids-Polyphenols	+++	+++	+	+	+++	+++
Tannins	+++		+		++	
Phytosterols	+++	+++	++	+	+++	+++
Phenols	+++	+++	++	++	+++	+++
Carotenoids	++		++		+++	
Fatty Acids	+	+	+	+	+	+
Steroids	++		-		+++	

+ = Present, − = Absent, ++ = Shows moderate concentration, +++ = Shows high concentration
Merge cells have powder values

Table 3. Quantitative determination of plant constituents.

Sr. no.	Quantitative determination of phytochemicals	Unit	Form	Experimental plants					
				Clove		Kolvengi		Karhi Patta	
				A	M	A	M	A	M
1.	Total flavonoids content (TFC)	Mg/g	Extract based	14.99	19.26	12.36	15.27	13.27	16.24
2.	Total phenolic content (TPC)	Gallic acid mg (GAE)/g		20.66	26.01	28.51	33.51	19.24	29.22
3.	Estimation of tannins	Tannic acid equivalent (mg/g)		16.62	18.13	14.35	15.11	12.09	12.48
4.	Determination of alkaloid content	Mg/g bismuth nitrate		133 mg/g		300 mg/g		90 mg/g	
5.	Selenium analysis	Selenium mg/l	Powder based	2.222		1.521		2.592	

A = Aqueous extract, M = Methanol extract.

In average values, order ranking with respect to standard was perceived as Karhi Patta < Kolvengi < clove < ascorbic acid in methanol and aqueous extracts with a bit difference in values as in Fig. 3. Clove and Kolvengi have the ability to donate electrons, so they can eliminate metal ions (Ananthi and Chitra 2013). Lastly, total antioxidant ability was assessed. Average antioxidant capacity of different plant extract was found to proliferate in clove, Kolvengi and then Karhi Patta. Strong antioxidant capacity of clove, almost reaching close to ascorbic acid was observed at 5000

µg/ml concentration as shown in Figure 4 (i) and (ii). Highly significant results were observed under regression modelling.

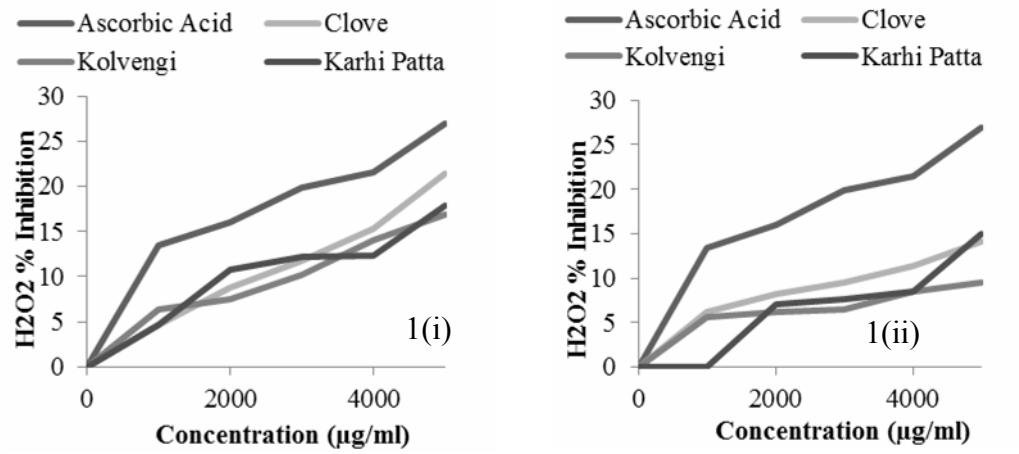


Fig. 1. Graph showing % hydrogen peroxide radical scavenging in plants with respect to ascorbic acid. (i) Methanol extract regression: Clove - 09825, Kolvengi - 0.9729, Karhi Patta - 0.8793. (ii) Aqueous extract regression: Clove - 0.9868, Kolvengi - 0.9266, Karhi Patta - 0.8316.

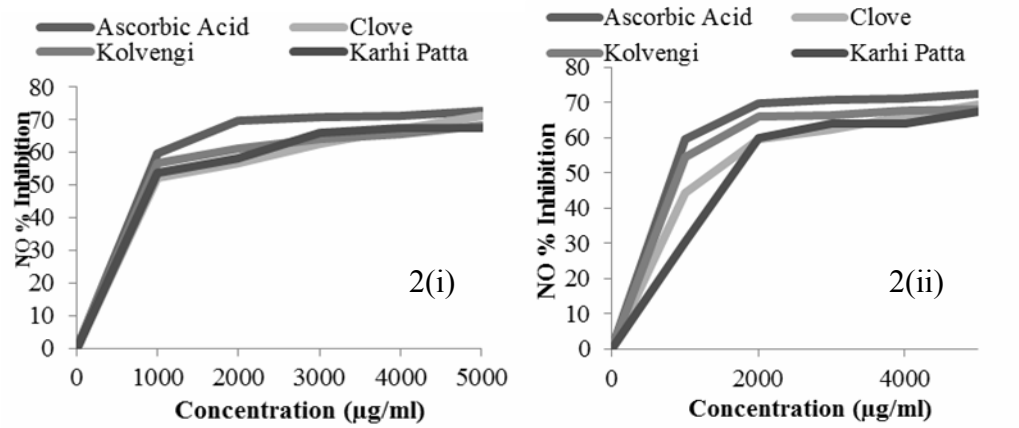


Fig. 2. Graph showing % nitric oxide radical scavenging activity of methanol and aqueous extract of selected plants with respect to ascorbic acid. (i) Methanol extract regression: Clove - 0.996, Kolvengi - 0.9599, Karhi Patta - 0.8524. (ii) Aqueous extract regression: Clove - 0.8468, Kolvengi - 0.6343, Karhi Patta - 0.6593

The harmful effects of noise, which induces oxidative stress in mice (mammals) at an alarming rate and to utilize plants for ultimate reduction of noise stress were also studied. The morphological effects observed after the noise introduction were decreased locomotion, loss of appetite, rubbing and scratching of paws. Franssen *et al.* (2004) reported that noise may cause sleep disturbances, behavioural changes, stress, annoyance and several diseases. Body weights were assessed before and after noise exposure and plants treatment. Body weight of control group was almost same. Negligible (0.133%) growth was observed. Mice exposed with noise lost their weight and mean values changed from 28.72 g to 27.94 g. Although this was a slight decrease but

such weight loss was developed due to noise effects and weakening of body defence. Experimental groups that were treated with plant extract showed body weight maintenance due to healing and oxidative stress mediator effects of plant antioxidants as shown in Fig. 5.

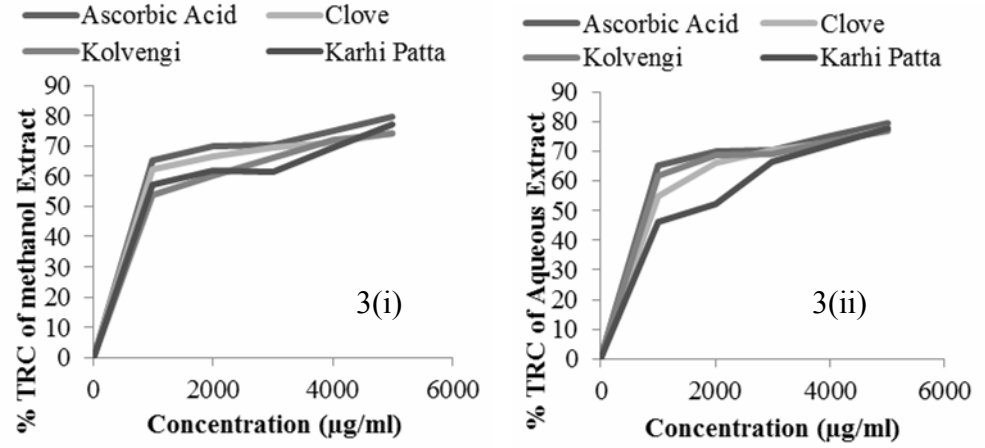


Fig. 3. Graph showing % in total reduction capability of plants with respect to ascorbic acid. (i) Methanol extract regression: Clove - 0.9786, Kolvengi - 0.9726, Karhi Patta - 0.9025. (ii) Aqueous extract regression: Clove - 0.9226, Kolvengi - 0.9726, Karhi Patta - 0.9715.

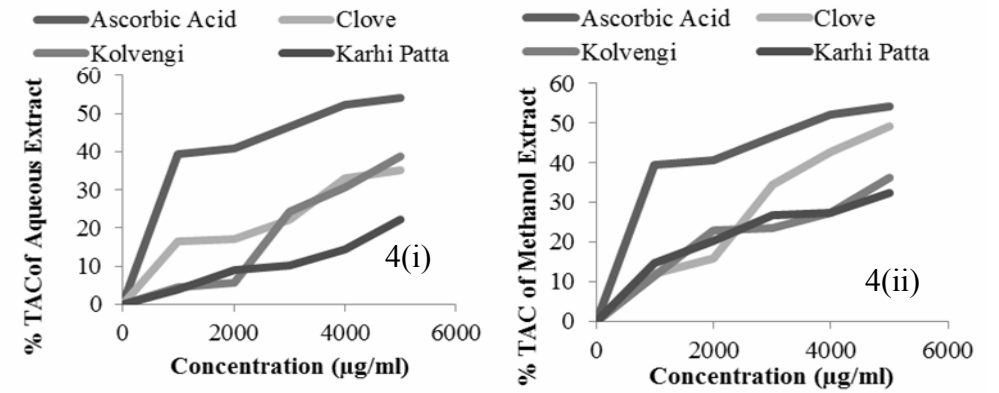


Fig. 4. Graph showing % total antioxidant capacity of plants with respect to ascorbic acid. (i) Methanol extract regression: Clove - 0.9575, Kolvengi - 0.9139, Karhi Patta - 0.9533. (ii) Aqueous extract regression : Clove - 0.9137, Kolvengi - 0.9449, Karhi Patta - 0.9417).

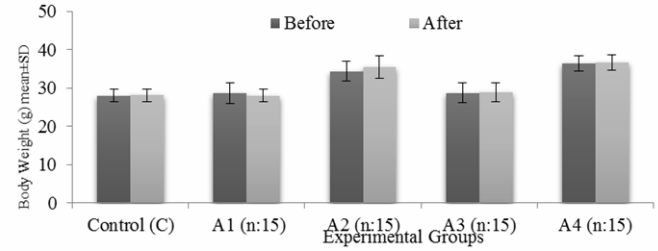
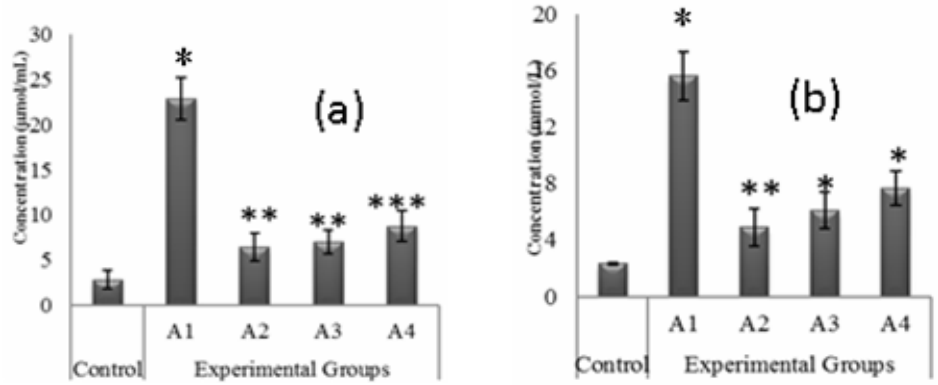


Fig 5. Upright and inverted bar graph showing % decrease/increase of body weights of control and experimental groups of albino mice.

Estimation of effects of noise on body cells revealed that mice of noise treated group without plant extract treatment showed significant high level of MDA ($p < 0.05$), when compared with control group. Mice those were treated with extract showed lowest values in A_2 group and high values in A4 but less than A_1 (Fig 6a). Similarly, Derekoy *et al.* (2001) found the same results at 100 dB. Studies provided information regarding increase in MDA in workers dealt with noisy machinery. Noise aggravates the level of oxidative stress and hearing loss. High level of MDA is directly proportional to cell membrane damage. Clove group showed minute level of MDA, which was due to its ability to remove hydroxyl radical to water by transferring electrons. It



* = Significant difference ($p < 0.05$), ** = More significant difference ($p < 0.01$)
*** = Highly significant difference ($p < 0.001$)

Fig. 6. Bar graph showing (a) level of malondialdehyde (MDA) and (b) Estimation of nitrite in tissue homogenate.

was also due to the presence of alkaloids, which has the potential to reduce MDA (Rafieian, 2013). It was observed under this study that under noise condition nitrite was generated. Nitrite concentration was detected at highest level in A_1 group which was only treated with noise. In control group it was almost negligible, but in groups A_2, A_3 and A_4 slight levels of nitrite were notified when compared with control as shown in Fig. 6b. All selected plants displayed a significant antioxidant potential with slight differences to regularize body defence against lipid peroxidation and nitrite levels under noise conditions. They will be useful to mitigate the effects of noise in workplace.

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