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Proximate Composition and Antioxidant Potential of Fruits of *Zanthoxylum Alatum* Roxb. (Tooth Ache Tree) and its Acceptability Assessment in Food Products

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ABSTRACT

Proximate composition of whole dried fruits of Zanthoxylum alatum, its seed and seed coat were analyzed and essential oil of seed coat was extracted and evaluated for total phenolic and antioxidant content. To assess acceptability of seed coat powder as a flavouring agent, different concentration of roasted seed coat powder were incorporated in selected Indian foods. Organoleptic evaluation of products was done by panel members. Results revealed that crude protein of whole dried fruits, seed and seed coat were 2.9%, 2.1% and 2.6% respectively. Essential oil extracted from seed coat exhibited good antioxidant activity. Food products prepared with roasted seed coat powder were highly acceptable as compare to control and scores were significantly higher at ($p>0.01$) paired t- test.

Key Words: *Zanthoxylum Alatum*, Tooth Ache Tree, Proximate Composition, Antioxidant, Total Phenol, Organoleptic Evaluation.

INTRODUCTION

The genus *Zanthoxylum* from family Rutaceae has 200 species, among them *Zanthoxylum alatum* Roxb. locally known as '*Timur*" which is an armed, erect shrub or a small tree (6 m tall or more), with dense foliage, found in the valleys of sub-tropical Himalayas. It also grows in the Eastern Ghats in Orissa and Andhra Pradesh at 1200m and the lesser Himalayan range in the northeastern part of India (e.g., Naga Hills, Meghalaya, Mizoram, and Manipur).

It is used as antiseptic, disinfectant, deodorant (Shinwariet et al. 2006)[1] antipyretic and anti diarrheal (Ahmad et al. 2006)[2]. It is also consider as a tonic, carminative, condiment, stomachic, anthelmintic (Verma and Khosa 2010)[3], Insecticidal (Tiwary et al. 2007)[4], toothache reliving, abortifacient, anti-fertility agent (Shah and Khan 2006)[5]. It also improves speaking power and increase saliva secretion (Ahmad et al. 2006)[6]. Fruits and seeds of this plant are used in fever, dyspepsia and skin diseases (Khare 2007)[7].

Fruits of *Zanthoxylum* and their pericarps are used as a peppery spice in both sweet and savory preparations. It is one of the traditional ingredients in the Chinese spice mixture five-spice powder along with star anise, fennel, clove, and

cinammon. It has a unique aroma and flavour that is not hot or pungent like black, white or chili peppers. Instead, it has slight lemony overtones and creates a tingly numbness in the mouth. In India, localities of *Kuamaon* region use *Timur* fruit in the form of condiments, spices and medicine.

In the preparation of certain traditional dishes, the use of *Timur* as a flavouring agent or spice is very popular. Ground, roasted *Timur* is used to make infused oil and is also paired with salt to make a flavorful seasoning. In view of several medicine properties of *Timur* and its unique taste, flavor and mouth feel the present study reports proximate composition and antioxidant of seeds of *Zanthoxylum alatum* along with its usage in various food products

MATERIALS AND METHODS

Procurement of plant material

Dried samples of *Timur* were procured from Krishi Vigyan Kendra of Pittoragarh district, Uttarakhand. Samples were cleaned well and seed and seed coat of whole *Timur* were separated. Whole *Timur* fruit (T), seed (S) and seed coat (SC) were weighed and transferred to poly bags and sealed in polythene pouches.

Proximate Analysis

All the three samples i.e. T, S and SC were analysed in triplicate for proximate composition. Samples of T, S and SC were ground using electronic food grinder before analysis and transferred to plastic containers. Moisture content was estimated by oven dry method. Protein content was measured by Kjeldahl procedure of total nitrogen using standard methods. Fat content was extracted from samples with petroleum ether (40-60) by Soxhlet apparatus, while ash content was measured by charring the sample in hot plate and placing in muffle furnace at 600° C for at least 3 hours then cooled in desiccators and weighed. The ash was almost white or greyish white in colour. Crude fibre was determined by treating oil-free sample by sulphuric acid (0.26 N) and potassium hydroxide (0.23 N) solution in refluxing systems, followed by oven drying and muffle furnace incineration (AOAC 1995)[8].

Extraction of essential oil

The seed coats were thoroughly washed twice with distilled water, ground in electronic grinder were subjected to hydrodistillation for 4 h in Cleavenger apparatus. The essential oil was separated and collected in a sterilized glass vial. Water traces from the essential oil were removed by adding anhydrous sodium sulphate. The essential oil was stored at 4 °C in dark till used.

Determination of total phenol content

Total phenol content of the essential oil of *Timur* seed coat was determined spectrophotometrically using Folin-Ciocalteu reagent (Gholivand et al. 2010)[9]. A solution (0.1 ml) containing the 1000 µg oil was mixed with 46 ml of distilled water in a volumetric flask. 1 ml Folin-Ciocalteu reagent was added and the mixture was thoroughly shaken using electronic shaker and left for 3 min to allow completing reaction. 3 ml aqueous solution of 2% Na_2Co_3 was then added. The solution was incubated for 4 h at room temperature. At the end of the incubation period, the absorbance of each mixture was measured at 760 nm. The same procedure was also applied to standard solutions of gallic acid (0-1000µg/0.1ml) and a standard curve was obtained.

Total phenolic content of the oil was obtained by comparing the absorbance value of oil at 760nm with standard curve and expressed as µg gallic acid equivalent/ mg of oil.

Absorbance = 0.0011 x gallic acid (µg) + 0.13

Antioxidant activity of *Timur* essential oil

Free radical scavenging activity of the essential oil of *timur* was measured by recording the extent of bleaching of a DPPH solution is mixed with a solution from purple to yellow (Prakash et al. 2011)[10]. During the DPPH radical assay, DPPH solution is mixed with a substance that has a capacity to donate the hydrogen atom giving rise to reduced form 1,1 diphenyl-2-picrylhydrazine (non radical). Different concentration of essential oil was added to 0.004% DPPH solution in methanol (5ml). After 30 min incubation at room temperature, the absorbance was measured against a blank at 517nm using spectrophotometer. Butylated hydroxytoulene (BHT)

and Butylated hydroxyanisole (BHA) (2.0 - 10 µg/ml) were used as positive controls. Scavenging of DPPH free radical with reduction in absorbance of the sample was taken as a measure of the antioxidant activity. The IC_{50} of the test positive control, which represented the concentration that caused 50% neutralization of DPPH radicals, was calculated from the graph plotting percentage inhibition against concentration, while the test sample was represented as percent inhibition.

$$I\% = (A_{blank}\text{-}A_{sample}/A_{blank}) \times 100$$

Preparation of food product from *Timur* seed coat powder (TSCP)

Four selected Indian food products i.e. lentil soup, *chilli paneer*, *besan sev* and *Punjabi chole* were developed in the laboratory. Different amounts (100-500 mg) of TSCP were incorporated in lentil soup, *chilli paneer*, *besan sev* and *Punjabi chole* where as standard recipe served as control. Standardization of TSCP at different levels (100-500mg) in products was done to obtain reproducible results. A group of 10 panel members were selected by threshold test (Swaminathan 1987)[11]. Acceptability test was conducted using 9-point Hedonic scale. A score card was developed to assess sensory characteristics such as colour, flavour, appearance, texture, taste and overall acceptability of the food products. Panel members were asked to assign a score using 9 point hedonic scale provided in the score card.

Lentil Soup

Lentil soup refers to a variety of vegetarian and meat soups made with lentils. The soup may consist of green, brown, red, yellow or black lentils, with or without the husk. Dehulled yellow and red lentils disintegrate in cooking, making a thick soup. In the present investigation a vegetarian lentil soup was prepared with the addition of TSCP and varying levels of TSCP to be added in per serving of soup. Ingredients for lentil soup included 15g Lentil *dhal*, 50g tomato, 10g onion, 3g salt, 1g turmeric powder, 5g butter, 1 bay leaf, 4 no. black pepper and 100-500mg TSCP.

Chilli Paneer

Chilli Paneer Recipe is a classic Indo Chinese dish that is inspired with the Asian sauces using Indian ingredients. It is a very popular appetizer in restaurants and parties and very easy to make as well. It can serve with other Indo Chinese Dishes like a Chilli Coriander Fried Rice. Different concentration of TSCP was standardized in per serving of Chilli paneer. Ingredients included 30g paneer, 30g corn flour, oil for frying, 20g onion, 5g ginger, 4 cloves garlic, 25g capsicum, 1 green chilli, 100mg -500mg TSCP.

Besan Sev

Besan sev, a variety of *farsan* or crunchy fried snacks made from Bengal gram flour. It is commonly used of snack found in every household. In the present study Besan sev was prepared with varying levels of TSCP (100-500mg) per serving. Ingredients included 50g gram flour, 15ml oil, 2g salt, 1g turmeric powder, 1g red chilli powder, 500mg omum, 100-500mg TSCP and oil for frying.

Punjabi chole

Punjabi chole also known as *chole masala* or *channay* is a popular *Punjabi* dish in Indian and Pakisthani cuisine. The main ingredient is chickpeas. It is fairly dry and spicy with a sour citrus taste. It is often eaten with a type of fried bread and is known as *bhature* as well as *kulcha* and *poori*. The different concentration of TSCP was standardized in per serving *chole*. Ingredients used for *Punjabi chole* was 30g chickpeas, 50g tomato, 30g onion, 4 cloves garlic, 3g ginger, 5g salt, 3g coriander seeds, 2g cumin seeds, 4 black pepper, 1 cloves, 1 cinnamon, 1g turmeric powder, 3g red chilli powder and 100-500 TSCP.

Statistical Analysis

Experimental results were statistically analysed by mean $\pm$ standard deviation of three parallel measurements. Significant differences between means were determined by paired *t*-test using a Microsoft Excel (Microsoft Office- 2007, India) statistical analysis program and $p < 0.01$ was considered as significant.

RESULTS AND DISCUSSION

Proximate Composition

The seed and seed coat was separated out from whole *Timur* (50g) to know the proportion of seed and seed coat. The values obtained for seeds were 22.993g (46%) and seed coats were 26.986g

(54%). Table 1 showed proximate composition of whole *Timur* (T), seed (S) and seed coat (SC). The data indicate that moisture content of T, S and SC was 12.6%, 10.7% and 17.8% respectively. Fat content of T was 11.3 % while S and SC contained 19.5% and 6.90%. The data showed that there was a wide variation in fat content of whole *Timur* when compared to its separated parts i.e. seed and seed coat. Protein content was found to be 2.9% in T, 2.1% in S and 2.6% in SC. The result indicates that protein content was higher in SC as compared to T and S. The values for ash content of T and SC was similar i.e. 7.0% and 7.1% however it was slightly higher in S (7.4%). Fibre content of T, S and SC was 1.4%, 0.6% and 2.3% respectively.

Extraction of essential oil

The essential oil of seed coat of *Timur* was light yellow in colour and its yield was 3.5% (V/W) during hydro-distillation. Seeds of *Zanthoxylum armatum* DC, yielded 1.2% (W/W) of the essential oil on hydrodistillation. The yield or variation of composition of essential oil obtained from the same species may have resulted from the environmental, developmental, genetic or some other differences. Production conditions, variety, cultivars or population were some factors that also affect the yield and composition of essential oil (Singh 2003)[12].

The standard curve of gallic acid for the measurement of total phenolic content showed in fig.1. The total phenolic content (TPC) of the essential oil obtained by comparing the absorbance value of oil at 760nm to the standard curve was found to be 28.91±0.56 µg/mg. The high phenolic content of the oil may be responsible for the higher antioxidant activity as scanvenger of the free radicals. Phenolic compounds are also very important plant constituents because their hydroxyl group confers scavenging ability (Dorman et al. 2003[13]; Wattenberg et al. 1989[14]; Zheng et al. 1992[15]).

Antioxidant activity

The free radical scavenging investigations measured by recording the extent of bleaching of DPPH from purple to yellow, showed that IC_{50} values of synthetic antioxidants used as positive controls was 9.7 µg/ml for BHT and 6.8 µg/ml for BHA whereas *Timur* essential oil has strong antioxidant activity at 100µl and 200µl showed 67.81% and 80.92% scavenging activity respectively. Essential oil of *Z. alatum* at a concentration of (20µg/ml) exhibited

Table 1: Proximate Composition of Timur, seed and seed coat (Mean + SD)

Nutritional parameters	Whole Timur (T)	Seed (S)	Seed coat (SC)
Moisture (g)	12.6 + 0.42	10.7 + 1.07	17.8 + 1.44
Fat (g)	11.3 + 0.30	19.5 + 1.59	6.90 + 0.85
Protein (g)	2.9 + 0.14	2.1 + 0.24	2.6 + 0.43
Ash (g)	7.0 + 0.06	7.4 + 0.12	7.1 + 0.13
Fibre (g)	1.4 + 0.09	0.6 + 0.2	2.33 + 0.29

Table 2: Scores of Organoleptic characteristics of developed food product using TSCP (100-500mg)

Food products	Control	100 mg	200 mg	300 mg	400 mg	500 mg
Lentil soup	7.46	7.76	8.2*	8.5*	8.18*	7.84*
Chilli paneer	7.44	7.92*	8.22*	8.38*	8.62*	8.64*
Besan Sev	7.66	8.02*	8.32*	8.52*	8.72*	8.44*
Punjabi chole	7.48	7.72	7.92*	8.1*	8.08*	8.44*

*p< 0.01 as compared to respective controls using Paired t-test

98.5% of antioxidant activity which is significantly higher than the oleoresin, BHA and BHT (Singh 2003). *Z. alatum* with its antioxidant activity was expected to protect against OH mediated damage of deoxyribose (Batool et al. 2010)[16].

Food product prepared from *Timur* seed coat powder

Acceptability of four selected Indian food products i.e. lentil soup, *chilli paneer*, *besan sev* and *Punjabi chole* prepared with addition of 100-500 mg were evaluated by panel of judges. Results of organoleptic evaluation of all the four food products were depicted in table 2. The Paired t-test showed overall acceptability of lentil soup incorporated with timur powder was significantly increased as compared to that observed in the control samples to

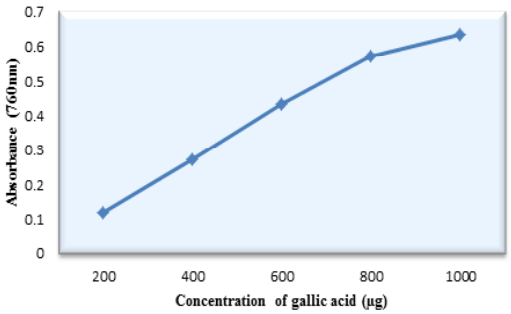

Fig. 1: The standard curve of gallic acid for the measurement of total phenolic content

a level of 300mg of incorporation, beyond which it was not acceptable due to its high flavor and pungency. The Chilli paneer was prepared with addition of *timur* powder from 100-500mg was evaluated by panel of judges. It was found that overall scores for acceptability of Chilli paneer was significant higher (p>0.01) at 500mg of incorporation. Whereas *besan sev* prepared with incorporation of TSCP was highly acceptable upto level of 400mg. However, scores for overall acceptability were highly significant at 500mg of incorporation of *timur* powder in *Punjabi chole* which is indicative of the association between the two. Bhotiya tribal community of Uttarakhand uses *timur* fruit in the form of condiments, spices and medicine. They used *timur* as a flavouring agent or spice in certain traditional dishes. Soup made from the dried fruit (known as *hag) is* consumed by the entire family to keep warm during winter. A *chutney* (like a sauce), locally known as *dunkcha,* is also a popular food item in this community (Kala et al. 2005)[17]. In Nepal, *timur* is used in the popular foods momo, thukpa, chow mein, chicken chilli and other meat dishes. It is also widely used in homemade pickles.

Along with food uses, *timur* have several medicinal properties. It is used in curing various common ailments such as toothache, common cold, cough, and fever, as it is believed to give warmth to the body. Fresh or dry fruit is pressed over the affected tooth and is kept in position till it loses its pungency helps in curing decay. Recently people have also started to use powder made from the dried fruit for cleaning teeth. Common stomach complaints are treated with *timur* soup. The fruits and seeds are employed as an aromatic tonic in dyspepsia, carminative, stomachic, anthelmintlc and expelling roundworms. The volatile oil is employed as an antidiarrehal, antiseptic, deodorant and anticataerhal. The oil has a good tenacity and is appreciated for its fixative qualities.

CONCLUSION

It can be concluded from present study that essential oil of *timur* had large amount of phenolic compounds, exhibits high antioxidant and free radical scavenging activities. Ground and roasted seed coat powder of *timur* act as a good flavouring agents and it was highly acceptable in selected Indian food products.

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REFERENCES

1. Shinwari, Z. K., Watanabe, T., Rehman, M., Yoshikawa, T., A pictorial guide to medicinal plants of Pakistan. *KUST, Kohat*., p. 400 (2006).
2. Ahmad, S., Ali, A., Beg, H., Dasti, A. A., Shinwari, Z. K., Ethnobotanical studies on some medicinal plants of Booni valley, district Chitral, Pakistan. *Pak. J. Weed Sci. Res.,* **12** (3): 183-190 (2006).
3. Verma, N., Khosa, R. L., Hepatoprotective activity of leaves of *Zanthoxylum armatum* DC in CCl4 induced hepatotoxicity in rats. *Indian J. Biochem. Biophys.,* **47**: 124-127 (2010).
4. Tiwary, M., Naik, S. N., Tiwary, D. K., Mittal, P. K., Yadav, S., Chemical composition and larvicida l activities of the essential oil of *Zanthoxylum armatum* DC (*Rutaceae*) against three mosquito vectors. *J. Vector Borne Dis.,* **44**: 198-204 (2007).
5. Shah, G. M., Khan, M. A., Checklist of Medicinal Plants of Siran Valley, Mansehra, Pakistan. *Ethnobotanical Leaflets*, **10**: 63-71 (2006).
6. Khare, C.P., Indian Medicinal Plants. An Illustrated Dictionary. Springer-VerlagBerlin Heidelberg, p. 730 (2007).
7. A. O. A. C., Official Method of Analysis, 16th edn, Association of Official Analytical Chemists, Washington DC, (1995).
8. Gholivand, M. B., Rahimi-Nasrabadi, M., Batooli, H., Ebrahimabadi, A. H., Chemical composition and antioxidant activities of the essential oil and methanol extracts of *Psammogeton canescens*. *Food Chem. Toxicol.,* **48**: 24-28 (2010).
9. Prakash, B., Shukla, R., Singh, P., Mishra, P. K., Dubey, N. K., Kharwar, R. N., Efficacy of chemically characterized *Ocimum gratissimum* L. essential oil as an antioxidant and a safe plant based antimicrobial against fungal and aflatoxin $_{B1}$ contamination of spices. *Food Res. Int.,* **44**: 385-390 (2011).
10. Swaminathan, M., Sensory methods of analysis of foods. Food science, chemistry and experimental foods. The Bangalore Printing and Publ. Co. Ltd, Bangalore, India; 286 (1987).
11. Singh, G., Kapoor, I. P. S., Singh, P., De, Heluani, Carola, S., De, Lampasona, Marina, P., Catalan-Cesar, A. N., Chemistry and Antioxidant Properties of Essential Oil and Oleoresins Extracted from the Seeds of Tomer (*Zanthoxylum armatum* DC). *Int. J. Food Prop.,* **16**: 288-300 (2003).
12. Dorman, H. J. D., Kosar, M., Kahlos, K., Holm, Y., Hiltunen, R., Antioxidant properties and composition of aqueous extracts from *Mentha* species, hybrids, varieties, and cultivars. *J. Agricul. Food Chem.,* **51**: 4563-4569 (2003).
13. Wattenberg, L. W., Sparnins, V. L., Barany, G., Inhibition of N-nitrosodiethylamine carcinogenesis in mice by naturally occurring organosulfur compounds and monoterpenes. *Cancer Res.,* **49**: 2689-2694 (1989).
14. Zheng, G. O., Kenney, P. M., Lam, L. K., Anethofuran, carvone and limonene: potential chemo preventive agents from dill weed oil and caraway oil. *Planta. Medica.,* **58**: 338-341 (1992).
15. Batool, F., Sabir, S. M., Rocha, J. B. T., Shah, A.H., Saify, Z.S., Syed, D.A., Evaluation of antioxidant and free radical scavenging activities of fruit extract from *Zanthoxylum alatum*: a commonly used Spice from Pakistan. *Pakistan J. Bot.,* **42**: 4299-4311 (2010).
16. Kala, C. P., Farooquee, N. A., Dhar, U., Traditional uses and conservation of through social institutions in Uttaranchal Himalaya, India. *Conservat. Soc.,* **3**: 224-230 (2005).