Phytochemical and free radical scavenging activity of underutilized plant, *Cordia dichotoma*

Neha Singh[1*], Vidhu Yadav[1] and Diksha Kinger[1]

ABSTRACT

Cordia dichotoma (*C. dichotoma*) fruit is a copious source of phytochemicals. The present communication deals with the phytochemical and free radical scavenging activity of the extract derived from fruit of underutilized plant (*C. dichotoma*) using four different solvents (methanol, ethanol, petroleum ether and aqueous). Highest extraction yield was obtained with methanol (14.4%) whereas petroleum ether displayed least value (7.1%). Quantitative estimation of phytochemicals revealed that methanol extract contained highest concentration of phenols (2.20 mg GAE/g), tannins (1.32 mg TA/g), flavonoids (2.08 mg QE/g) and antioxidant activity (94.3%) compared to other extracts prepared by using solvents like ethanol, petroleum ether and aqueous. Incorporation of *C. dichotoma* powder into product like chutney can enrich daily food with phytochemical and antioxidants.

Keywords: *Phytochemicals, Antioxidants, Cordia dichotoma, Medicinal plant*

1. Introduction

Cordia dichotoma (C. dichotoma/Indian cherry/lasura/ borla) belongs to the Boraginaceae family (Kuppast*et al*, 2006). It is one of the traditional deciduous plants that grows worldwide and from ancient times it is known for its medicinal properties. All the parts of the plant (leaves, fruit, bark and seed) have antidiabetic, antiulcer, anti-inflammatory, analgesic, antidysentery, hepatoprotective, diuretic and laxative activities (Jamkhande*et al*, 2013). The entire plant of *C. dichotoma*is edible and used as food. The seeds of the *C.dichotoma* contain taxifolin which exhibit DPPH free radical scavenging activity at a concentration of 100µg/mL (Mahaswetа*et al*, 2014). Various physico-chemical studies also showed that mucilage is acceptable, suitable and has potential to be used as an additive in novel drug delivery systems for controlled drug delivery (Gupta *et al*, 2015).

C. dichotoma tree is found in variety of forests which are ranging from dry deciduous forests of Rajasthan to the moist deciduous forests of Western Ghats and tidal forest in Myanmar. In addition to this, it is also found in Maharashtra in moist monsoon forest (Patel *et al*, 2011). Soon after the flowering, fruits are developed that ripen from June to August in northern part of India and before May in southern parts of India. The research showed that fruit part of the plant contained macronutrients mainly, carbohydrates (18g), proteins (35g), and fats (37g) and several minerals Ca(55mg), P(275mg), Zn(2mg), Fe(6mg), Mn(2mg), Cr(0.2mg), Cu(1.6mg/100gm) (Deshmukh et al, 2011).

C. dichotoma may be efficaciously used to cure ulcerative colitis (Ganjare*et al*, 2011), inflammations (Shahapurkar& Jayanthi, 2011), ulcers (Shah*et al*, 2011), wounds (Kuppast & Nayak, 2006), bacterial infection (Konka*et al*, 2014), fertility issues (Sharma*et al*, 2015), etc

Phytochemicals are biologically active ingredients which possess therapeutic properties and are contemplated as medicine or drug (Shakaya, 2016). These phytochemical constituents protect plants against microbial infection or infestation by pest and also help in curing and healing certain human ailments (Nostro*et al*, 2000). Phyto-chemicals are not vital nutrients for sustaining human life but play a cardinal role in preventing or treating some of the diseases such as cancer, tumor, diabetes etc. The seed part of the plant contained various bioactive compounds such as α-amyrins, betulin, octacosanol, lupeol-3- rhamnoside, β-sitosterol, hentricontanol, taxifolin-3,5- dirhamnoside, hesperitin-7-rhamnoside and fatty acids (palmitic acid, stearic acid, arachidic acid, behenic acid, oleic acid and linoleic acid, flavonoid glycosides (robinin, rutin, rutoside, datiscoside and hesperidin), phenolic derivative (chlorogenic acid and caffeic acid) (Hussain&Kakoti. 2013). Similarly, one more study quoted various other chemical constituents in fruits and leaves are Pyrrolizidine alkaloid, coumarins, flavanoids, saponins, terpenes, sterol, arabinoglucan, D-glucose (67.6%), L-arabinose (13.2%), Quercetin and quercitrin (Thirupati *et al*, 2008).

Antioxidants are compounds that prevent the formation of free radicals or interrupt the propagation of free radical by

1. *Department of Food & Nutrition & FT, Institute of Home Economics, University of Delhi*
* *Corresponding Author* ✉ *nehanics2013@gmail.com*

Received: 30 June, 2023
Available online: 20 September, 2023

one of several mechanism. Even, at small concentration, antioxidants have ability to inhibit the oxidation process and thus play different physiological role in the body. Antioxidants from plants act as a radical-scavengers that help in converting the oxygen radical (highly reactive species) to less reactive species. Antioxidants act as a first line of defense against free radical damage and are critical for maintaining optimum health. The methanolic extract of *C. dichotoma* possess good antioxidant activity (Rawat, 2013).

Medicinal plant offer alternative remedies with tremendous opportunities and more than half of the world's population still rely largely on traditional remedies for health care. One of the main reasons for this is that they do not have access to or could not afford the present health care services. Also, traditional herbs and plant parts have been shown to have medicinal value that can be used to prevent, alleviate or cure several human diseases. All the parts of the *C.dichotoma* plant have antioxidant as well as phytochemical properties. It has pharmaceutical properties and medicinal value. Fresh fruit of *C. dichotoma* contain gum that has thickening, emulsifying, gelling, suspending, binding, lubricating properties.

The objective of the present study was to utilize lesser known plant (*C.dichotoma*) to prepare a food product that is nutritionally rich and also supplement medicinal properties. The present study focused on the measurement of phytochemical and free radical scavenging activity of dried fruit of *C.dichotoma*using different solvents for extraction because both the free radical scavenging activity and phytochemical properties of plant differs with solvent.

2. Methodology

2.1 Procurement and preparation of raw material

The raw material for this study was procured from local market of north-west Delhi. The dried fruit of *C. dichotoma* was homogenized to fine powder by using grinder and then stored in airtight container.

2.2 Preparation of crude extract

The extraction was performed by hot percolation method using Soxhlet method. Wherein, 15gm of dried fruit powder was placed into a glass thimble then extracted with 200ml of different solvents separately (ethanol, methanol, petroleum ether and acetone). The extraction proceeds till the solvent in siphon tube of Soxhlet apparatus become colorless. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure using rotary evaporator. The crude extract was kept in refrigerator at 2-4^{o}C for future use. The extraction yield was calculated by following formula:

*% Extractive Value = W1/W2*100*

W1: Weight of dry extract residue left after solvent removal

W2: Weight of the dry sample

2.3 Physicochemical Analysis

Moisture content, Acid – insoluble ash, Water – soluble ash, and Crude fiber were determined by methods suggested by Gogakar *et al*. 2017.

2.4. Phytochemical screening of fruit extract

Different extracts were screened for the presence of various phytoconstituents such as alkaloids, phenols and tannins, flavonoids, saponins, steroids, terpenoids, proteins, and carbohydrates.

2.5 Quantitative analysis of phytochemicals

2.5.1 Total Phenols

The Folin-Ciocalteu Spectrophotometric method was used for determination of total phenolic content in crude extract .To a 25 ml volumetric flask, 1 ml of extract and 9 ml of distilled water was taken. One ml of Folin-Ciocalteu phenol reagent was added to this mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate solution was added to the mixture. The volume of the same was made to 25 ml with distilled water. A standard curve was developed using different concentrations of gallic acid (20, 40, 40, 60, 80 and 100 µg/ml). Incubated for 90 minutes at room temperature and the absorbance values for test and standard solutions were noted against blank at 550 nm with a UV /Visible Spectrophotometer. Total phenol content was expressed as mg of GAE/gm of extract (Hanane *et al*, 2010).

2.5.2 Tannins

Colorimetric estimation of tannins is based on the measurement of blue colored formed by the reduction of phosphotungstoolybdic acid by tannin like compounds in alkaline solution.The oxidizing agent used was Follin-Denis reagent. To 0.1 ml of diluted extract (10mg/ml) ,0.5ml of folin denis reagent (to 75ml of water ,10g sodium tugnstate ,2 g of phosphomolibidic acid and 5 ml of 85% phosphoric acid were added. The mixture was refluxed for 2 hr , cooled to 25 0C and dilute to 100ml of distilled water ,then 1ml of saturated sodium carbonate was added and the volume was made upto 10 ml with distilled water. For color development, the sample was kept for 30 min and the absorbance was measured at 760 nm against blank by UV-VIS spectrophotometer. The results were expressed as mg TA/g of extract (Rangana ,1997).

2.5.3 Flavonoids

The Aluminium Chloride Colorimetric Assay was employed for quantifying flavonoids in the crude extracts. One ml of each extract and 4 ml of distilled water were taken into a 10ml volumetric flask. To this flask, 0.3 ml of 5 % sodium nitrite was added and after 5 min, 0.3ml of 10 % aluminium chloride was mixed. Thereafter, 2 ml of 1M sodium hydroxide was treated after 5min. and the content was diluted to 10 ml with distilled water. A standard curve was prepared with quercetin solution (20, 40, 60, 80 and

100 µg/ml) as the procedure described earlier. The absorbance readings were recorded for test and standard solutions against blank at 510 nm in UV/Visible spectrophotometer. The total flavonoid content was expressed as mg of QE/g of extract (Hanane *et al*, 2010).

2.6 DDPH radical scavenging activity

Radical scavenging activity of the extract from the fine dried powder was determined. A 0.1 ml of extract solution was mixed with 3.9 ml methanol and 1 ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution. The dispersion was kept in dark at ambient temperature for 30 min and reduction in the absorbance was read at 517 nm. The scavenging activity was calculated by following equation (Moon & Shibamoto, 2009).

*DPPH radical scavenging = [1-(Absorbance 517nm control/ Absorbance 517nm sample)]*100*

2.7 Incorporation of fine powder of dried *C. dichotoma* fruit

C. dichotoma fruit powder was incorporated in tomato chutney (prepared using standard procedure) at different levels keeping all other ingredients the same. Two variants were prepared one with 15% fruit powder and other with 30% fruit powder. After preparation, both the variations were evaluated by semi-trained panel.

2.8 Sensory evaluation

Overall acceptability of product was rated on Hedonic Rating Scale (5-point scale). Panelist consisting of 25 members (that included faculty members and students) analyzed the product. Panelist analyzed the product for color & appearance, texture, taste, aroma and overall acceptability.

2.9 Statistical Analysis

The data obtained through the various tools and techniques were tabulated on the master sheets and one-way ANOVA was used in the study to establish significance.

3. Results and Discussion

3.1 Physicochemical Analysis

The dried powder was tested for moisture content, total ash and crude fiber. The extraction was carried out using different solvents. Results of physicochemical parameters are summarized in Table 1.

Moisture content – During storage dried powder gains moisture from the surrounding and facilitate the growth of microbes which ultimately deteriorate the quality of the final product. Therefore, moisture content is an indicator of freshness and stability of powder on storage. The moisture content of dried fruit powder was 13.4% which was higher than the one observed by previous workers (9.3%) (Deore and Namdeo, 2013).

Ash content – Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a food sample. The total Ash value of the plant materialrepresent the amount of minerals and earthy materials attached to the plant material. Ash value is animportant quantitative standard and criterion to identify the purity and identity of crude drugs especially in the powder form (Patni*et al*, 2012). Acid –insoluble ash is a part of total ash that measures the amount of silica present, specifically as sand and siliceous earth while water soluble ash is the water soluble portion of the total ash. The analytical results showed that total Ash value was 12.9%while acid – insoluble and water-soluble ash value of fruit powder were 4.06% and 3.33% respectively. The ash values were comparatively higher as observed by Deore and Namdeo (Deore and Namdeo, 2013).

Crude fiber – Crude fiber refers to the residue of a feed that is insoluble after successive, boiling with dilute acid and alkali. It is composed largely of cellulose (60-80%) and lignin (4-6%) along with some mineral matter. Crude fiber is helpful in curing or preventing coronary heart diseases, constipation and some type of cancer (Gogakar*et al*, 2017). The crude fiber content of dried fruit powder of *C. dichotoma* was found to be 47.3%.

Table 1: *Physicochemical Parameters of C. dichotoma Fruit*

S. No.	Parameters	Mean %
1.	Moisture	13.4 ± 0.22
2.	Total Ash value	12.9 ± 0.20
3.	Acid - insoluble ash	4.06 ± 0.15
4.	Water- soluble ash	3.33 ± 0.07
5.	Crude fiber	47.3 ± 0.25

* The results are presented as mean ± SE, n=3

3.2 Extraction Yield

The Dried fruit powder was used for extraction with a series of solvent in their increasing order of polarity i.e., methanol, ethanol and petroleum ether by Soxhlet apparatus whereas aqueous extract was obtained by reflux extraction.

The results displayed in Table 2, shows that extraction yield was increased with the increase in polarity of the organic solvent. Therefore, highest extraction yield (14.4%) was obtained from extraction with methanol whereas petroleum ether displayed least extractive value (7.1%). These results are in agreement with the results obtained by Rawat*et al* (2013), where percentage yield of different extracts of *C. dichotoma* leaves were evaluated and the results showed that extraction yield was highest with methanol followed by chloroform and petroleum ether had least.

Moreover, methanol, ethanol and aqueous extracts were brown in color and petroleum ether extract was light green color. All the extracts showed sticky consistency except methanol which had greasy consistency. Chigayo *et al* (2016) also conclude in their study that 80% methanol can be the best solvent for extraction.

Table 2: *Extraction yield of different extracts of fruit of C. dichotoma*

Sr. No.	Type of solvent	Color	Odour	Consistency	Extractive value%
1.	Methanol	Brown	Characteristics	Greasy	14.4
2.	Ethanol	Brown	Characteristics	Sticky	8.7
3.	Petroleum ether	Light Green	Characteristics	Sticky	7.1
4.	Aqueous	Brown	Characteristics	Sticky	11.9

3.3 Screening of Phytochemical Compounds

Extracts of dried powder were analyzed for qualitative and quantitative phytochemical properties. The results of qualitative analysis of phytochemicals are shown in Table 3.

Table 3: *Preliminary phytochemical screening of C. dichotoma fruit extracts*

Sr. No.	Phytoconstituents	Methanol	Ethanol	Petroleum ether	Aqueous
1.	Glycosides	++	++	-	+
2.	Flavonoids	++	+	-	+
3.	Phenol & tannins	++	+	+	++
4.	Steroids	+	+	-	+
5.	Saponins	-	-	-	-
6.	Terpenoids	-	-	-	-
7.	Alkaloids	+	+	-	+
8.	Proteins	-	-	-	++
9.	Carbohydrates	+	+	-	+

++ Deep coloration; + Slight coloration; - Not detected

Preliminary phytochemical screening of *C. dichotoma* fruit extract showed the presence of various bioactive components. The results provide evidence of the presence of glycosides, flavonoids, phenol & tannins, steroids, alkaloids and carbohydrates in methanol, ethanol and aqueous extracts. Petroleum ether was the only solvent in which most of the tested bioactive components were absent except phenol and tannins. The results also revealed the absence of saponins, terpenoids and proteins in methanol, ethanol and petroleum ether extracts. The results of present investigation are in accordance with Deorde and Namdeo (Deorde and Namdeo, 2013). Besides this, Mulani*et al* (2013), connocated that methanol can be used as a solvent for the purpose of extraction of phytochemicals as methanol extracts of the plant show positive results and more deep coloration for number of chemical tests.

Major outcomes of the present investigation revealed that the fruit extract of *C. dichotoma* contained high concentrations of health-enhancing phytochemical constituents. Alkaloids, tannins, flavonoids and phenolic compounds are some of the bioactive chemical constituents of the plants that bear medicinal value and are responsible for producing a definite physiological action on the human body (Okwu, 2001).

3.4 Quantitative Analysis of Phytochemicals

Phytochemicals from fruits and vegetables show protective effect against many diseases as there is an inverse relation between consumption of fruits and vegetables and many type of cancers such as stomach, colon, cancer, breast (Kris-Ethertone*t al*, 2002). ICMR also recommended consumption of fruits and vegetables in generous amount (400g/d) to protect against certain chronic disorders (Gey, 1993). Hence, phytochemical analysis is considered as effective approach in discovering bioactive profile of plants for therapeutic importance.

3.4.1 Total Phenol Content (TPC)

The total phenol content of the extracts of *C. dichotoma* fruit were analyzed in terms of gallic acid equivalent (mg GAE/g of extract). methanol extract contained highest total phenol content (2.20) followed by aqueous extract (0.86) and ethanol extract (0.06) whereas petroleum ether showed the least total phenol content (0.02). In general, the results of the present study revealed that extraction yield of total phenols was higher with methanol from dried fruit powder than the other solvent systems tested. These results arein accordance with those reported by Nandedkar *et al*, (2013).

3.4.2 Total Tannins

The values obtained for the concentration of tannin content are expressed as gallic acid equivalent (mg of TA/g of extract). The highest concentration of tannins was observed in methanol extract i.e. (1.32 mg TA/g) which is in accordance to that observed by Nandedkar and coworkers (Nandedkar *et al*, 2013). The total tannin content in aqueous, ethanol and petroleum ether extracts were 1.09 mg TA/g, 0.95 mg TA/g and 0.25 mg TA/g, respectively (Table 6). Therefore, the present investigation demonstrated that methanol is the best solvent for extraction of tannins from *C. dichotoma* whereas efficiency of petroleum ether to extract tannin from plant material is least.

3.4.3 Total Flavonoid content (TFC)

Flavonoids inhibit or kill many bacterial strains, inhibit important viral enzymes, such as reverse transcriptase and protease, destroy some pathogenic protozoans (Havesteen, 2002). The biological functions of flavonoids are linked to their potential cytotoxicity and their capacity to interact with enzymes through protein complexation (Williams*et al*, 2004).

The daily intake of flavonoids with normal food, especially fruit and vegetables, is 1-2 g but modern physicians suggest higher intake of fruits and vegetables for the treatment and prevention of many chronic diseases because of their ability to inhibit specific enzymes, to stimulate hormones and neurotransmitters and to scavenge free radicals (Havesteen, 2002).

The result demonstrated that fruit of *C. dichotoma* is rich in flavonoids. The flavonoid content of the dried fruit powder extract in different solventswereanalyzed in terms of quercetin equivalents (mg QE/g). The results outlined in table 6 stated that highest total flavonoid content was observed in methanolic extract (2.08 mg QE/g) which is in agreement with the observations by Mulani and coworkers (Mulani*et al*, 2013). After methanolic extract, highest flavonoid content was sustained by aqueous extract (0.79 mg QE/g) and then ethanolic extract (0.05 mg QE/g) whereas flavonoids were absent in petroleum ether extract.

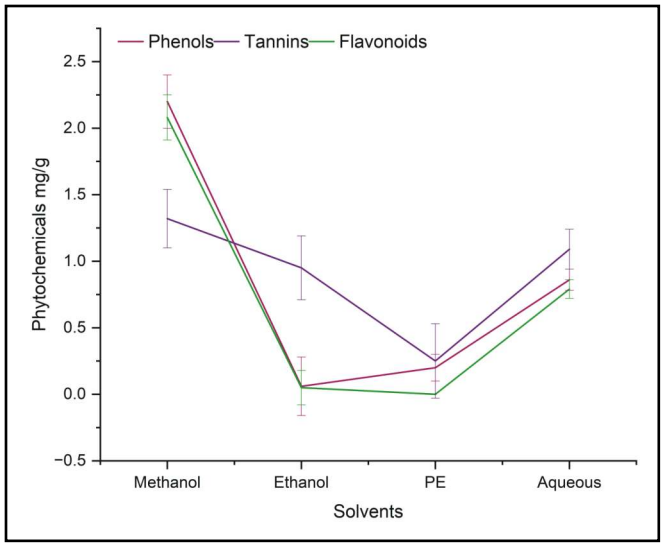

Fig.1: *Phytochemical constituents present in different extracts*

3.4.4 DPPH Radical Scavenging Activity

DPPH radical scavenging activity of the dried fruit powder extracts in different solvents was evaluated in the present investigation and the results are shown in the Table 4. The results demonstrated that highest free radical scavenging activity was showed by methanolic extract (94.3%) followed by aqueous extract (95.5), ethanol extract (71%) and petroleum ether extract (53%). So, from this we conclude that methanol and aqueous extract show similar free radical scavenging activity whereas petroleum ether showed least radical scavenging activity.

3.4.5 Incorporation of dried powder of *C. dichotoma* fruit

In the present study, fine powder of dried *C. dichotoma* fruit was incorporated in tomato chutney to develop *C.dichotoma*chutney. The dried fruit of *C. dichotoma*is shown in Fig. 1 and Fig.2 shows *C. dichotoma* powder. The products prepared by adding *C. dichotoma* powder are shown in Fig. 3. Sample A is the product that is prepared with 30% *C.dichotoma* fruit powder and the variation made with 15 % *C.dichotoma*fruit powderis shown as sample B.

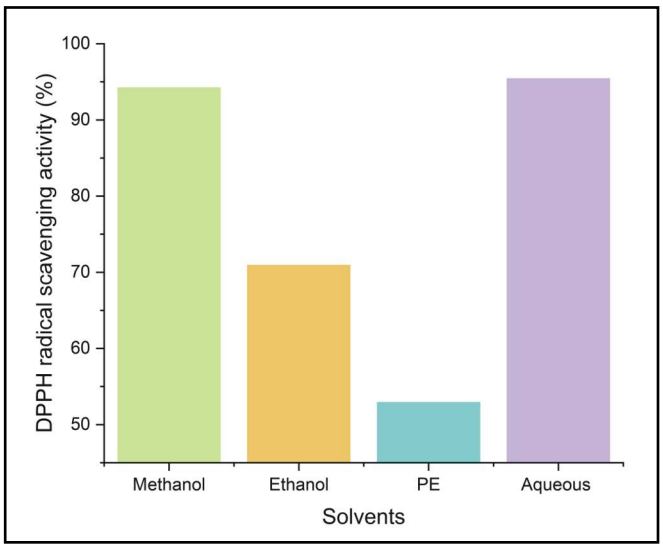

Fig.2: *DPPH radical scavenging activity of different extracts*

Fig. 3: *Dried fruit of C. dichotoma*

Fig. 4: *C. dichotoma powder*

Fig. 5: *Products prepared by adding C. dichotoma powder*

3.4.6 Sensory evaluation of developed product

Sensory evaluation is important for testing the acceptability of the prepared product. The products were evaluated for color &appearance, texture, color, taste, aroma and overall acceptability using a five-point scale. Two variants of product were evaluated along with control for the sensory characteristics in order to find the best one for all parameters.

Table 4: *Sensory Evaluation of C. dichotoma Chutney*

Parameters	Mean Sensory Score		
	Control	30%variation (Sample A)	15% variation (Sample B)
Appearance	$3.96^{b} \pm 0.79$	$3.6^{a} \pm 1$	$3.94^{b} \pm 0.80$
Color	$4.16^{b} \pm 0.80$	$3.36^{a} \pm 0.95$	$4.25^{c} \pm 0.91$
Texture	$3.68^{b} \pm 0.94$	$3.48^{a} \pm 1.04$	$3.86^{c} \pm 0.91$
Aroma	$3.72^{b} \pm 0.84$	$3.64^{a} \pm 0.75$	$3.84^{c} \pm 0.86$
Taste	$3.84^{b} \pm 0.89$	$3.68^{a} \pm 1.22$	$3.94^{b} \pm 0.98$
Overall acceptability	$3.62^{b} \pm 0.75$	$3.56^{a} \pm 1.08$	$3.70^{b} \pm 0.86$

Mean with different superscripts with in a row are significant ($p<0.05$)

For sensory parameters like appearance, taste, and overall acceptability, the mean sensory scores for the variation made with 30% *C.dichotoma* fruit powder (sample A) were significantly ($p<0.05$) different from control and the variation made with 15 % *C.dichotoma* fruit powder (sample B).

Whereas, for parameters like color, texture and aroma, the mean sensory scores of all the three products (control, Sample B and Sample A) varied significantly ($p<0.05$). So, from this data we can conclude that sample B was acceptable in terms of sensory characteristics and was more liked by the consumers.

3.4.7 Consumer preference for chutney

The preference of consumers for chutney made with *C. dichotoma*fruit powderwas analysed. Data revealed that 70% of consumers preferred sample B (15% variation) whereas percent liking of sample A and control was 10% and 20% respectively. When consumers were asked how often would they like to have the products prepared with *C. dichotoma*fruit powder, 70% said daily and 30% said occasionally.

4. Conclusion

The present investigation elucidated that the plant *C. dochotoma* exhibited good phytochemical and antioxidant capacity. Amongst the four solvents (aqueous, methanol, ethanol, and petroleum ether), the highest extractive yield was observed with methanol as a solvent while petroleum showed the least. Similarly, phenol, tannin, and flavonoid content were found to be high in methanol extract.

Therefore, powder prepared from the dried fruit of *C. dichotoma* could be utilized as a source of phytochemicals and can be incorporated into products like chutney that are consumed regularly.

5. References

1. Chigayo K, Mojapelo PEL, Mnyakeni-Moleele S, MisihairabgwiJM.Phytochemical and Antioxidant Properties of Different Solvent Extracts of *Kirkiawilmsii* tubers.Asian Pacific Journal of Tropical Biomedicine 2016; 6(12): 1037-1043.
2. Deore SR, Namdeo AG. Pharmacognostic Evaluation and Preliminary Phytochemical Screening of *CordiaDichotoma*Forst Fruits. International Journal of Pharma and Bio Sciences 2013; 4(2): 961-965.
3. Deshmukh AS, Setty CM, Badiger AM, Muralikrishna K S. *Cordiadichotoma* Gum: A Functional Polysaccharide for Pharmaceutical Applications. International Journal of Current Pharmaceutical Research 2011; 3: 10-13.

4. Ganjare AB, Nirmal SA, Patil AN. Use of Apigenin from *C. dichotoma*in the Treatment of Colitis.Fitoterapia2011; 82:1052-1056.
5. Gey KF. Prospects for Prevention of Free Radical Diseases, Regarding Cancer and Cardiovascular Diseases.British Medical Bulletin 1993; 49: 679-699.
6. Gogakar Y, Manda M, Eda P, Malladi N, Chappala A, G.Hema C, Chandaka M. Estimation of Crude Fiber Content in Spices And fruits. Indo American Journal of Pharmaceutical Sciences 2017; 4 (10): 3864-3868.
7. Gupta S, Parvez N, Sharma PK. Extraction and Characterization of *Cordia Dichotoma* Mucilage as Pharmaceutical Adjuvant. World Journal of Pharmaceutical Research 2015; 4(2): 1299-1308.
8. Hanane El Hajaji, NadyaLachkar, KatimAlaoui, YahyanCherrah, Abdellah Farah, AbdesslamEnnabili. Antioxidant Properties and Total Phenolic Content of Three Varieties of Carob Tree Leaves from Morocco. Records of Natural Products, 2010; 4(4): 193-204
9. Havesteen BH. The Biochemistry and Medical Significance of the Flavonoids. Pharmacology and Therapeutics 2002; 96(2-3): 67-202.
10. Hussain N, Kakoti BB. Review on Ethno botany and Psychopharmacology of *Cordiadichotoma*. Journal of *Drug Delivery* and Therapeutics 2013; 3(1): 110-113.
11. Jamkhande PG, Barde SR, Patwekar SL, Tidke PS. Plant profile, Phyto-chemistry and Pharmacology of *Cordiadichotoma* (Indian cherry): a review *Asian Pacific* Journal of Tropical Biomedicine 2013; 3: 1009–1012.
12. Kiranmai,M., Kumar,M., Mohammed, I. Comparison of Total Flavanoid Content of *Azadirachtaindica* Root Bark Extracts Prepared by Different Methods of Extraction. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2011; 2: 254-26.
13. Konka R, Murali K.T, Siddhartha M, Thota S.P, Kiran R.K. *C. dichotoma* Crude Extracts: Potent Source of Natural Antibacterial and Antioxidant Agents. International Journal of Advanced Research 2014; 2 (2): 65-73.
14. Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE and Etherton TD. Bioactive Compounds in Foods: Their Role in the Prevention of Cardiovascular Disease and Cancer. American Journal of Medicine 2002: 113: 71–88
15. Kuppast IJ, Nayak PV. Wound Healing Activity of *Cordiadichotoma*Forst. F. Fruits. Indian Journal of Natural Products and Resources 2006; 5(2):99-102.
16. Lal G. Preservation of Fruits and Vegetables.Indian Council of Agricultural Research, New Delhi 1959, p: 208.
17. Mahasweta R, Kumar B, Kumar N, Patel A, Kumar B. Antioxidant Activity of Taxifolin Obtained from Methanolic Extracts of *C.dichotoma*L inn. Seeds. International Journal of Pharmaceutical Sciences and Research 2014; 5(7):2896-2901.
18. Maisale AB, Attimarad SL, Haradigatti DS, Karigar A. Anthelmintic Activity of Fruit Pulp of *Cordiadichotoma*.International journal of Ayurveda and pharmaceutical research, 2010; 1(2): 597.
19. Moon, JK, Shibamoto T, Antioxidant Assays for Plant and Food Components. Journal of Agricultural and Food Chemistry 2009; 57(5): 1655-1666.
20. Mulani RM, Solankar BM, Nandedkar PH. Phytochemical Estimation and Antimicrobial Activity of Methanolic and Acetone Extracts of *CordiaDichotoma* F. Asian Journal of Phytomedicine and Clinical Research 2013; 1(4): 211-217.
21. Nandedkar PH, Mulani RM, Solankar BM. Phytochemical Estimation and Antimicrobial Activity of Methanolic and Acetone Extracts of *CordiaDichotoma* F. Asian Journal of Phytomedicine and Clinical Research 2013; 1(4): 211-217.
22. Nostro A, Germanò MP, D'angelo V, Marino A, CannatelliMA.Extraction Methods and Bioautography for Evaluation of Medicinal Plant Antimicrobial Activity. *Letters* in *Applied Microbiology* 2000; 30: 379-384.
23. Okwu D. Evaluation of the Chemical Composition of Indigenous Species and Flavouring Agents. International Journal of Pure and Applied Sciences and Technology 2001; 7(3): 455- 459.
24. Patel AK, Pathak N, Trivedi H, Gavania M, Patel M, Panchal N. Phytopharmacological properties of *Cordiadichotoma*as a Potential Medicinal Tree: An Overview. *International Journal* of *Life science* and *Pharma* Research 2011; 1(1): 40-51.
25. Patni S, Sah AN, Meena H, Pandey HK, Manchanda A. Physico-chemical, Phyto-chemical and Elemental Analysis of Stem Bark and Roots of *Berberisasiatica*. Advances in Applied Science Research 2012; 3 (6): 3624-3628.
26. Rangana M. Manual of Fruit and Vegetable Product Analysis.MacGraw- Hill, New Delhi 1997p: 643
27. Rawat S, Saini R and Sharma A. Phytochemical study and antimicrobial activities of cordiadichotoma. International Research Journal of Pharmacy 2013., DOI: 10.7897/2230-8407.041212
28. Seidel V. Initial and Bulk Extraction of Natural Products Isolation. Methods in Molecular Biology 2012: 27-41.
29. Shah D, Prasad N, Limbani B. Gasroprotective and Antiulcer Effect of *C. dichotoma*.International Research Journal of Pharmacy 2011; 2(9): 70-72.
30. Shahapurkar AA, Jayanthi. Drug Neomycin Release from *Cordiadichotoma* Transdermal Film and Anti-inflammatory Activity. International journal of pharmaceutical research 2011; 2(9): 107-109.
31. ShakyaAK.Medicinal Plants: Future Source of New Drugs. International Journal of Herbal Medicine 2016; 4(4): 59-64.

32. Sharma P, Manjusha, Rani S, Malhotra H, Nitesh, Deswal S, Singh S. Antifertility Potential of Hydroalcoholic Extract of *C. dichotoma* G Forst. Leaves: A Folklore Medicine Used by Meena Community in Rajasthan State in India. Asian Pacific Journal of Reproduction 2015; 4(2): 100-105.
33. Shi J, Nawaz H, Pohorly J, Mittal G, Kakuda Y, Jiang Y. Extraction of Polyphenolics from Plant Material for Functional Foods – Engineering and Technology. Food Reviews International 2005; 21(1): 139-166.
34. Thirupathi K, Kumar SS, Raju VS, Ravikumar B, Krishna DR, Mohan GK. A Review of Medicinal Plants of The Genus *Cordia*: Their Chemistry and Pharmacological Uses. Journal of Natural Remedies 2008; 8(1): 1-10.
35. Tiwari B, Brunton N, Brennan C. Handbook of Plant Food Phytochemicals. Chichester: Wiley-Blackwell 2013.
36. Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: Antioxidants or Signalling Molecules? Free Radical Biology and Medicine 2004; 36(7): 838-849.

❖ ❖ ❖