International Journal of Advanced Technology in Engineering and Science
Vol. No.4, Issue No. 05, May 2016
www.ijates.com
ijates
ISSN 2348 - 7550

THE EFFECT OF TWO DIFFERENT EXTRACTION METHODS FOR ANTIOXIDANT ACTIVITY ON PETROLEUM ETHER EXTRACT OF LEAVES OF PLANT *CASEARIA TOMENTOSA*

Abha Shukla[1], Ritu Tyagi[2], Rishi Kumar Shukla[3]

[1,2]Department of Chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, Haridwar, Uttarakhand , (India)

[3]Department of Chemistry, Gurukula Kangri Vishwavidyalaya, Haridwar, Uttarakhand, (India)

ABSTRACT

In the present study the effect of maceration and soxhlet extraction from plant leaves of Casearia tomentosa were compared for phytochemical screening and antioxidant activity using petroleum ether as solvent. The phytochemical screening was evaluated using standard protocols and the antioxidant activity was performed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. The results of preliminary phytochemical screening in both extracts revealed the presence of terpenoids, steroids, phytosterol, fat and oil etc. But the extract obtained from soxhlet method showed higher antioxidant activity (IC_{50} value 280 µg/ml) than maceration (IC_{50} value 480 µg/ml). All these experimental analysis shows that the Casearia tomentosa leaves could be a potential source of natural antioxidant and may be used to prevent oxidative stress.

Keywords- Antioxidant activity, Casearia tomentosa, Free radical scavenging, Phytochemical, Terpenoids etc.

I INTRODUCTION

Extraction is an important step involved in the discovery of bioactive components from medicinal plants. Different extraction methods have been used to extract compounds from plant material such as Maceration, Soxhlet, Infusion, Digestion, Decoction, Percolation, Ultrasound extraction, supercritical fluid extraction etc. Biological activities of plant extracts showed significant differences depending upon the different extraction methods, emphasizing the importance of selecting the suitable extraction methods [1].

Antioxidants both endogenous and exogenous whether synthetic or natural can be effective in preventing free radical formation by scavenging them or by promoting their decomposition and suppressing many disorders [2]. Number of synthetic antioxidants such as BHT, BHA, propyl gallate, Tertiarybutylhydroquinone (TBHQ) have been added to food stuff [3].Although the synthetic antioxidant are efficient and cheap, there are some disadvantages because they are suspected of having some toxic parameter. Natural antioxidants play a key role in health maintenance and prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [4, 5, 6]. In the last few decades,the demand for natural antioxidants has been increased due to consumer concerns about the safety of synthetic antioxidants [7]. Therefore, search of natural antioxidants has been received much attention and efforts have been made to identify natural compounds that can act as suitable antioxidants to replace synthetic one.

One of such natural source is *Casearia tomentosa* belongs to the family *Salicaceae.* Different parts of *Casearia tomentosa* is traditionally claimed for its medicinal importance like in ulcers, dropsy, fissures, colic pain in the abdomen, malarial fever, tonsillitis pain, Skin disease, wounds, and in severe bone fractures as a plaster [8].The present investigation was undertaken to explore the effect of two different extraction methods on the antioxidant potential of this plant and to put forward the evidence of the fact that this plant is having good antioxidant activity.

II MATERIAL AND METHODS

2.1 Plant material

Casearia tomentosa leaves were collected from Lachhiwala forest Dehradun, Uttarakhand (India) in the month of August, identified and authenticated by Botanical Survey of India, (BSI) Dehradun with accession No.115689. A voucher specimen has been deposited in medicinal plants herbarium in Department of Chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya with register number 1/3. The collected leaves were washed, dried in shade and finally grinded to powdered form and stored in polythene bags for further use.

2.2 Chemicals and reagents

(DPPH) 1, 1-diphenyl-2-picrylhydrazyl (Sigma Aldrich), Ascorbic acid (Rankem, India),Petroleum ether (Merck), Ethanol (Merck). All the other solvents and chemical used were of analytical grade.

2.3 Extraction of *C. tomentosa* leaves

Two different extraction methods were followed to prepare crude extracts from leaves of C. tomentosa.

2.3.1 Maceration

Powdered plant material (150 g) was extracted with 1800mL petroleum ether for 72 hour at room temperature in a 3000ml conical flask, with occasionally shaking. The residue was extracted twice with fresh solvent and extracts combined. The above process was repeated until complete extraction [9].

2.3.2 Soxhlet

150 gm air dry powderd leaves of *Casearia tomentosa* was treated with 1250 mL of petroleum ether by soxhlet extraction technique for 18 hour until the solvent comes out of the extractor becomes pure and colourless [9].

Both extracts were concentrated to dryness under reduced pressure and controlled temperature using rotary evaporator. The collected leaves extract was stored in a refrigerator.

2.4 Phytochemical screening

The phytoconstituents present in petroleum ether extracts were analyzed by using standard qualitative methods [10, 11]. Extract was screened for the presence of biologically active compounds like alkaloids, flavonoids, tannins, glycosides, saponins, protein, terpenoids, steroids, fat and oil etc.

2.5 DPPH free radical scavenging assay

The free radical scavenging assay of petroleum ether leaves extracts of *Casearia tomentosa* was evaluated by stable DPPH free radical according to the method of Brand-Williams et.al with some modification [12]**.** A working solution of 0.004%was freshly prepared by dissolving 4 mg of DPPH in 100 ml of methanol. 1ml of each extract dilution of different concentration (1, 5, 10, 50, 100, 500, 1000 µg/ml) was added to 3 ml working solution of DPPH, Keep this reaction mixture in dark for 30 min.After 30 min the absorbance of the preparations were taken at 517 nm with an UV-VIS spectrophotometer which was compared with the corresponding absorbance of standard ascorbic acid of similar concentrations (1-1000 µg/ml). 1 ml of methanol with 3ml of working DPPH solution serves as blank. Then the % inhibition was calculated by equation-(1)

$$\%\text{Inhibition} = (\text{Absorbance of control} - \text{Absorbance of sample}) / (\text{Absorbance of control}) \times 100 \quad \text{-------(1)}$$

IC_{50} of extracts and standard ascorbic acid was calculated by graphical method by plotting % inhibition *vs* concentration.

2.6 Statistical Analysis

The experimental results are expressed as mean ± standard deviation of triplicate measurement and the results are processed using Microsoft Excel 2010 and sigmastat variance.

III RESULT AND DISCUSSION

2.7 Extractive yield

The extractive yield and consistency of petroleum ether extracts of *Casearia tomentosa* leaves was shown in Table 1

Table 1: Extractive yield of Petroleum ether leaves extracts of *Casearia tomentosa*

Serial no.	Extract	Color/ Consistency	% Yield (% w/w)
1.	Petroleum ether Soxhlet	Greenish Yellow/ Waxy	1.515
2.	Petroleum ether Maceration	Greenish Yellow/ Waxy	0.825

2.8 Phytochemical screening

The result for phytochemical screening of *Casearia tomentosa* leaves extracts are summarized in Table.2. Extracts from both extraction method (Soxhlet and Maceration) showed the presence of similar phytochemicals such as steroids, phytosterol, terpenoids and fats and oils etc. There is no difference between qualitative phytochemical profiles of two extraction methods.Out of these phytochemical, terpenoids are among the most wide spread groups of natural products. It is reported that plant derived terpenoids possess activities like antioxidant, anticancer, cytotoxic activity, anti-inflammatory, sedative etc [7]. Plant steroids also referred to as 'cardiac glycosides' are one of the most naturally occurring plant phytoconstituentsand numerous reports support their use as cardiac drugs and as antioxidant[7,13]. Beside these phytosterols was also present in this plant extract, which is also responsible for antioxidant activity [14]. Various reports support that plants fixed oil have variety of biological activity such as cytotoxic and antioxidant etc [15].

From above discussion, we can interpret that the presence of these phytochemicals in extracts shows medicinal importance of leaves of *C. tomentosa*.

Table 2: Phytoconstituents present in Petroleum ether leaves extracts of *Casearia tomentosa*

Phytoconstituents and Test performed		Petroleum ether Maceration extract	Petroleum ether Soxhlet extract
Alkaloids	Mayer's Test	-	-
	Wagner's Test	-	-
	Hager's Test	-	-

	Dragendroff's test	-	-
Flavonoids	**Alkaline test**	-	-
	Lead acetate test	-	-
	Shinoda's Test	-	-
	Sulphuric acid test	-	-
Tannins	**Ferric chloride test**	-	-
Carbohydrate	**Molisch's Test**	-	-
	Fehling's Test	-	-
	Benedict's Test	-	-
	Barfoed's test	-	-
Glycosides	**Keller-Killiani Test**	-	-
	Legal's Test	-	-
	Borntrager's test	-	-
Terpenoids	**Liebermann burchard test**	+	+
	Salwoski test	+	+
	Salwoski test (Triterpenes)	-	-

Steroids	**Liebermann burchard test**	+	+
Fat and Oil	**Saponification test**	+	+
	Filterpaper test	+	+
Saponin	**Foam test**	-	-
Protein and amino acid	**Millon's test**	-	-
	Ninhydrin	-	-
	Biuret	-	-
Phytosterol	**Salwoski test**	+	+
	Liebermann burchard test	+	+
Anthraquinone	**Benzene test**	-	-

+: Present, -: Absent

2.9 DPPH Free Radical Scavenging Assay

DPPH is a purple colored stable free radical; when reduced it becomes the yellow-colored diphenyl-picryl hydrazine. DPPH radicals react with suitable reducing agents and then electrons become paired-off and the solution loses colour stoichimetrically with the number of electrons taken up [16, 17,18].The DPPH radical scavenging activity of petroleum ether extracts of *C. tomentosa* was detected.Among Petroleum ether extracts of leaves of *C. tomentosa*, Soxhlet extract showed better DPPH radical scavenging activity (IC_{50} value 280± 0.011µg/ml) than Maceration(IC_{50} value 480± 0.010µg/ml). Thermo stable compounds extracted through hot extraction may be the reason for better antioxidant activity of this plant. The IC_{50} value for DPPH assay for two different extracts are shown in Table-3. Though these extracts showed good DPPH scavenging activity but it was less effective than standard Ascorbic acid (IC_{50} value 20±.260 µg/ml) but being natural product they are more safer than synthetic Ascorbic acid.The % inhibition is shown graphically in Fig.1.

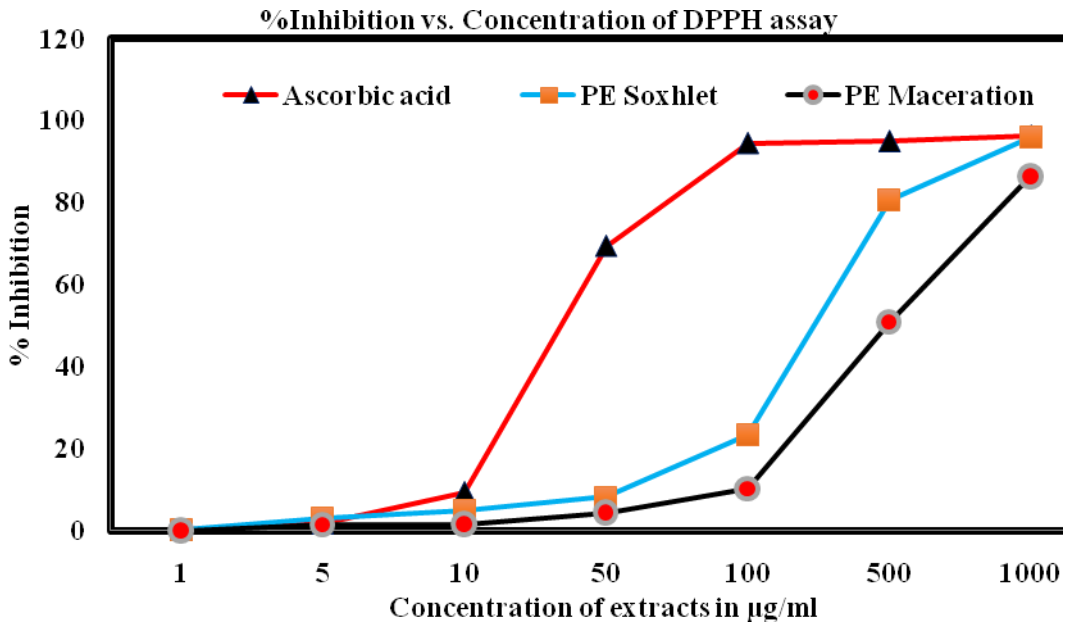

Fig1:- DPPH radical scavenging activity of leaves extracts of C*asearia tomentosa and* standard ascorbic acid.

III CONCLUSION

In the present study, two extraction methods were used to evaluate the phytochemical screening and antioxidant activity of petroleum ether extracts of plant *C. tomentosa* which revealed that different type of extraction methods had a big influence on antioxidant activity. Among Petroleum ether extracts of plant *C. tomentosa*, Soxhlet extract has greater extractive yield. In spite of similar preliminary phytochemecal, Soxhlet extract showed better antioxidant activity. From the result we conclude that this is due to thermo stable active compounds which are extracted out due to heat. Soxhlet extraction could provide not only better results than the maceration but also showed a significant advantage in extraction time and solvent consumption over the maceration [1].The findings of the present study suggest that *Casearia tomentosa* leaves could be a potential source of natural antioxidant that could have great importance as therapeutic agents in preventing or slowing the progress of aging and age associated oxidative stress related degenerative diseases.

IV ACKNOWLEDGEMENT

The authors are thankful to Department of Chemistry, Kanya Gurukula Campus, Gurukula Kangri Vishwavidyalaya, Haridwar for providing all the necessary facilities.

REFERENCES

[**1**] R. Muugan ,T. Parimelazhagan, Comparitive evaluation of different extraction methods for antioxidant and anti-inflammatory properties from Osbeckia parvifolia Arn.-An in vitro approach. *J king saud univ sci,26,,2014,267-275.*

[**2**] A.Shukla, S. Vats, R.K. Shukla,Preliminary phytochemical screening,Antibecterial and Nitric oxide Radical Scavenging activities of *Reinwarditia indica* leaves extract ,*Int J PharmTech Res, 5(4), 2013,1670-1678.*

[**3**] A.Shukla, S. Vats, R.K. Shukla, Phytochemical screening, Proximate Analysis and Antioxidant Activity of *Dracaena reflexa Lam.* Leaves, *Indian j pharma sci, 77(5), 2015, 640-644.*

[**4**] A. Shukla, R. Tyagi, S. Vats, R.K. Shukla, Total phenolic content, antioxidant activity and phytochemical screening of hydroalcoholic extract of *Casearia tomentosa* leaves, *J. Chem. Pharm. Res., 8(1),2016,136-141.*

[**5**] M.A.Jayasri, L. Mathew & Radha, A. . A report on the antioxidant activities of leaves and rhizomes of *Costus pictus* D. Don. *International Journal of Integretive Biology*. 5(1),2009,20-26.

[**6**] S.N.Uddin, M.A. Akond, S. Mubassara & M.N. Yesmin, Antioxidant and Antibacterial activities of *Trema cannabina. Middle-East Journal of Scientific Research*. 3,2009, 105-108.

[**7**] P. Moreira, M.A. Smith, X. Zhu, K. Honda, H.-G. Lee, G. Aliev and G. Perry, Since oxidative damage is a key phenomenon in Alzheimer's disease, treatment with antioxidants seems to be a promising approach for slowing disease progression. Oxidative damage and Alzheimer's disease: Are antioxidant therapies useful? News Persp, Drag (2005).

[**8**]K.T.Rao, V. Sree devi., A. Veerabhadrappa, M.K. Chetty, L.Ramesh , Pharmacognostical studies of *Guidonia tomentosa*, Indian Journal of Plant Sciences, 3(1), 2014,136-144.

[**9**] N. Raaman, *Phytochemical Techniques*, New India Publishing Agency, New Delhi, India (2006) p. 10

[**10**] WC Evans : *Trease and Evans' Pharmacognosy.*16th ed. Elsevier Health Sciences publishers. UK, 2009.

[**11**] J.B.Harborne. *Phytochemical Methods A Guide To Modern Techniques of Plant Analysis.* 4th ed. Springer publishers,1998.

[**12**]W.Brand-Williams, M E,Cuvelier, C. Berset, Use of a Free Radical Method to Evaluate Antioxidant Activity**,**,*Food Science and Technology,28,1995, 25-30* .

[**13**] A.D.Mooradian, Antioxidant properties of steroids, *The Journal of Steroid Biochemistry and Molecular Biology* , 45(6), 1993,509-511.

[**14**] T. Wang , K.B. Hicks, R. Moreau, Phytosterols, triterpene alcohols, and phospholipids in seed oil from white lupin, *Journal of the American Oil Chemists' Society*, 79(12), 2002, 1201-1205.

[**15**] F. Muna, Abushama, I. Hilmi Yasmin, M. Haidar AbdAlgadir, EltayebFadul and E. Hassan Khalid, Lethality and Antioxidant Activity of some Sudanese Medicinal Plants' Fixed Oils ,*European Journal of Medicinal Plants,4(5),2014,563-570.*

[**16**] M.S. Blois, Antioxidants determination by the use of a stable free radical , *Nature, 4617, 1958,1199–1200.*

[**17**]I.B. Afanasev, A.I. Dorozhko, A.V. Brodskii, V.A. Kostyuk, A.I. Potapovitch, Chelating and free radical scavenging mechanisms of inhibitory action of Rutin and Quercetin in lipid peroxidation , *Biochem. Pharmacol., 38, 1989,1763–1769.*

[**18**] R.K. Shukla, D. Painuly, A. Shukla, J. Singh, A. Porval and S. Vats, *In vitro* biological activity and total phenolic content of *Morus nigra* seeds, *J. Chem. Pharm. Res., 6(11), 2014, 200-210.*