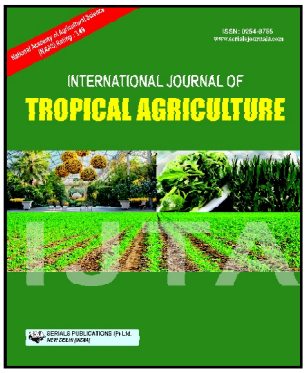

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Phytochemical Screening of Secondary Metabolites present in Fruit peels, *Ex-vitro* and *In-vitro* leaf extract of *Citrus macroptera* Montr.: An important crop with medicinal nutritional value

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Abstract: The genus Citrus belongs to the orange subfamily Aurantioideae of the family Rutaceae and order Sapindales which is one of the largest orders. *Citrus* species is grown in tropical and subtropical regions around the globe.*Citrus macroptera*Montr.is semi wild and categorized as an endangered species. It is economically significant and pharmacologically diverse medicinal species of North Eastern region of India. Various bioactive compounds, metabolites, plant pigments are frequently extracted to evaluate its effects on plant growth and productivity, stress related responses. It is a rich source of alkaloids, flavonoids and terpenoids due to which it gains importance in pharmaceutical industry. It possesses multivariate properties; oil extracted from this crop is utilized in perfume industry. The fruit peel of *C.macroptera* has significant cytotoxic, antimicrobial, anti-hypertensive, and antipyretic activities. Phytochemical screening of peel and leaf (*ex-vitro* & *in-vitro*) extracts in the different organic solvents like chloroform, ethanol and methanol revealed the presence of steroid, reducing sugar, terpenoid, phlobatannins and flavonoids. The peel extracts contain maximum of the phytoconstituents as compared to the leaf extracts and methanol extract showed the intense presence of phytochemicals as compared to ethanol and chloroform extract it can be concluded that from leaf also important phytochemicals can be obtained which can further be enhanced as it possesses high potential against various diseases and could serve as prime source of efficacious drugs.

Key words: Citrus macroptera, Pharmaceutical, flavonoids, phytochemical screening

INTRODUCTION

Citrus macroptera belongs to the Rutaceae family and it is native of the regions of Southeast Asia mainly Indonesia, Myanmar, Thailand, Papua New Guinea, Malayasia Sylhet Division of Northeastern Bangladesh and northeastern India mainly Manipur

and Assam (Carpenter & Reece, 1969; Dreyer & Huey, 1973). In Manipur, C. macroptera occur in the subtropical forests of North-east India and the foot hills of East Himalayas (Nair *et al.*, 1997; Dassanyake & Fosberg 1985). In Manipur, this wild orange is found in the Chandel District and Jiribam Sub-Division of Imphal East District (Nongalleima *et al.*, 2017).

Citrus fruits are known for their rich nutritional, dietary, medicinal and cosmetic properties and this species of Assam is also valued for its fruits of which the dried peels are used as a flavoring agent in the form of spice in for meat dishes, its juice is used in the treatment of diarrhea also the leaves are used for extracting oil which could be used in the perfume industry (*http://gsl.articlealley.com/an-introduction-of antidepressant activity. satkara-1932990.html*)

Plants possess the capability to produce a wide range of bioactive compounds, known as phytochemicals, which may act as natural antioxidant against free radical damage (Suffredini *et al.*, 2004). Scientists have examined the effect of various types of solvents, such as methanol, ethanol, chloroform and other alcohol, for the purpose of phytochemicals extraction from several parts of the plants, such as peels, leaves and seeds. Moreover, identification and classification of extracts is necessary because of its various benefits as the knowledge obtained makes pharmacological studies convenient .The mode of action of the plants producing effects can also be investigated if the active components of the plants are classified (Shukla & Tyagi, 2017).

Yip and Dallman (1988) and Dallman *et al.* (1980) revealed the contribution of the phytoconstituents of *C. macroptera*, such as polyphenols, flavonoids, and organosulfur compounds, towards its neuropharmacological effects. Ethanolic extract of *C. macroptera*fruit peels (EECM) were found to possess anxiolytic and antidepressant activities (Aktar & Foyjun., 2017). The peel extracts of Citrus macroptera is found to have cardio protective effects as it diminishes the oxidative stress in myocardium (Paul *et al.,* 2017). The present study is based on extracts of *C. macroptera* peel and leaves derived from different organic solvents such as methanol, ethanol and chloroform which would reveal the presence of phytochemicals that are responsible for medicinal and therapeutic bioactivities.

MATERIALS AND METHODS

Plant material

Fruits of *Citrus macroptera* were collected from the village of Chandel district of Manipur. The *ex-vitro* leaves were collected from the field grown plant which was also procured from ICAR Manipur center Lamphelpat, Imphal. The *in-vitro* leaves were collected from the plant tissue culture facility of Applied Plant Biotechnology laboratory of Amity University, Noida.

Preparation of Extract

Fruit peel and leaves (*in-vitro* and *ex-vitro*) were dried in hot air oven at 56°C. Dried rind sample was finely powdered using blender. The leaves samples were powdered using mortar and pestle. The ground samples were extracted in the three different organic solvents namely, methanol, ethanol, and chloroform in the ratio of 1:4.

PHYTOCHEMICAL SCREENING

The extracts were analyzed for the presence of phytochemicals: following tests were performed for the screening

Test for Steroids: 1ml of the extract was dissolved in 10 ml of the chloroform and equal volume of sulphuric acid was added by the sides of the test tube. If the upper layer turns red and sulphuric acid layer showed yellow with green fluorescence, which indicates the presence of steroids (Syed UJ *et al.*, 2017).

Test for reducing sugar: Mix 1 ml each of Fehling solution A and Fehling solution B and add few drops of extract into it in a test tube, keep the tube at 60 ^{O}C in water bath, the colour turns from blue to red which shows the presence of reducing sugar (Nandan & Vangalapati, 2015).

Test for Terpenoids: 1ml of extract was placed into the test tube and 0.4 ml of chloroform along with the 0.6 ml of concentrated sulphuric acid were added gently into the tube from the sides which gives reddish brown colour showing presence of terpenoids (Gotmare&Gade, 2018).

Test for Phlobatannins: 1% aqueous HCl is added to 1 ml of extract and the mixture is boiled which gives red precipitate indicating the presence of phlobatannins.

Test for Flavonoids: 1ml of 10% NaOH was mixed with 1 ml of filtrates, and the mixture was shaken vigorously for the development of yellow colour, which shows the presence of flavonoids (Syed UJ *et al*, 2017).

RESULTS AND DISCUSSION

The results of phytochemical screening on the ethanolic, methanolic and chloroform fruit peel extract of *C.macroptera* revealed that they were rich in phytochemical activity, as shown in Table 1, 2 & 3 where phlobatanins and flavonoids were highly present when extracted from methanol while steroid, terpenoid and phlobatanins were present moderately both in the ethanol and chloroform extract, also reducing sugar was present in trace amounts both in methanol and ethanol extract and in moderate amounts in chloroform extract, hence it was concluded that all the three organic solvents could be used for the extraction process but the best result could be obtained using methanol and ethanol extracts.

Similarly, the following tests for the phytochemical screening were performed using *ex-vitro* and *in-vitro* leaves where fair amounts of phlobatanins, terpenoids, flavonoids and reducing sugars were present in *ex-vitro* extracts of the organic solvents whereas trace amounts of terpenoids, flavonoids, and reducing sugar were present in the organic solvent extracts of *in-vitro* leaves. From table 1&3, it was concluded that for the phytochemical analysis of *ex-vitro* and *in-vitro* leaves methanolic and chloroform extract could be used.

Table 1
Phytochemical screening with Methanolic extracts of peel, *ex-vitro* and *in-vitro* leaves

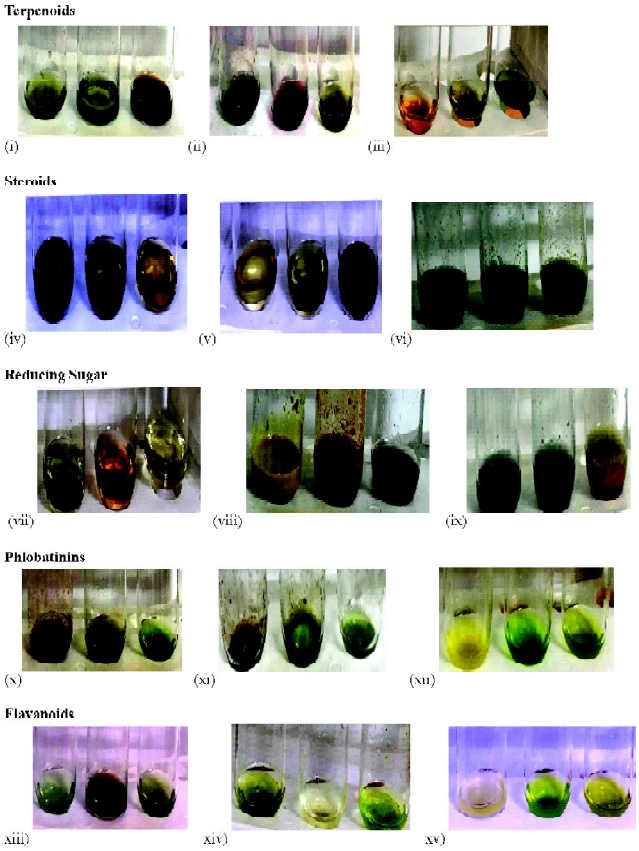
Test leaves	*Peel*	*Ex-vitro leaves*	*In-vitro*
Steroid	++	-	-
Reducing Sugar	+	-	-
Terpenoid	++	-	+
Phlobatanins	++++	++	-
Flavonoids	++++	-	+

Table 2
Phytochemical screening with Ethanolic extracts of peel, *ex-vitro* and *in-vitro* leaves

Test leaves	*Peel*	*Ex-vitro leaves*	*In-vitro*
Steroid	+++	-	-
Reducing Sugar	+	-	-
Terpenoid	+++	+	-
Phlobatanins	+++	-	-
Flavonoids	+	+	+

Table 3
Phytochemical screening with Chloroform extracts of peel, *ex-vitro* and *in-vitro* leaves

Test	*Peel*	*Ex-vitro leaves*	*In-vitro leaves*
Steroid	+++	-	-
Reducing Sugar	+++	+++	+
Terpenoid	+	+	+
Phlobatanins	++	-	-
Flavonoids	-	-	-



(i) Terpenoid test for methanol extract of *In-vitro* leaf, peel, *ex-vitro* leaf

(ii) Terpenoid test for ethanol extract of *ex-vitro* leaf, *In-vitro* leaf & peel

(iii) Terpenoid test for chloroform extract of *in-vitro* leaf, peel & *ex-vitro* leaf

(iv) Steroid test for methanol extract of *ex-vitro* leaf, *In-vitro* leaf & peel

(v) Steroid test for ethanol extract of peel, *in-vitro* leaf & ex -vitro leaf

(vi) Steroid test for Chloroform extract of *In-vitro* leaf, peel, *ex-vitro* leaf

(vii) Reducing sugar test for methanol extract of *in-vitro* leaf, *ex-vitro* leaf & peel

(viii) Reducing sugar test for ethanol extract of *in-vitro* leaf, *ex-vitro* leaf & peel

(ix) Reducing sugar test for Chloroform extract of *In-vitro* leaf, peel, *ex-vitro* leaf

(x) Phlobatannis test for Chloroform extract of peel, *ex-vitro* leaf & *In-vitro* leaf

(xi) Phlobatannis test for methanol extract of peel, *ex-vitro* leaf & *In-vitro* leaf

(xii) Phlobatannis test for ethanol extract of *In-vitro* leaf, peel, *ex-vitro* leaf

(xiii) Flavonoid test for methanol extract of peel, *ex-vitro* leaf & *In-vitro* leaf

(xiv) Flavonoid test for ethanol extract of peel, *ex-vitro* leaf & *In-vitro* leaf

(xv) Flavonoid test for Chloroform extract of *In-vitro* leaf, peel, *ex-vitro* leaf

CONCLUSION

Fruit peel, *ex-vitro* and *in-vitro* leaves were screened qualitatively for the presence of phytochemical constituents using different organic solvents. The content of steroid, reducing sugar, terpenoids, phlobatanins and flavonoids were quantified for all the extracts and it is concluded that presence of different secondary metabolites in *in vitro* leaf explants in methanol, ethanol and chloroform extracts. The presence of phytochemicals present in *in vitro* leaves can be exploited for large scale production through plant tissue culture technique which could further be used in pharmacological, food, oil and perfume industry.

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REFERENCES

Aktar K., Foyzun T. (2017). Phytochemistry and Pharmacological Studies of *Citrus macroptera*: A Medicinal Plant Review. *Evidence-Based Complementary and Alternative Medicine,* 9789802: 7.

Carpenter JB, Reece PC. (1969). Catalog of genera, species and subordinate taxa in the orange subfamily Aurantioideae (Rutaceae). *Beltsville, Md.: U.S. Department of Agriculture. Agricultural Research Service,* 34-106.

Dallman P., Siimens M., and Stekekl A. (1980). Iron deficiency in infancy and childhood. *The American Journal of ClinicalNutrition* 33:86–118.

Dassanyake, M.D., Fosberg, F.R. (1985). A Revised Handbook of the Flora of Ceylon, Vol. V. *Smithsonian institution / Amerind Publishing Co, New Delhi* ISBN 13: 9789061910657

Dreyer DL, Huey PF (1973). Coumarins of *Citrus macroptera*. Phytochemistry; 12(12):3011-3013.

Gotmare S., Gade J. (2018). Orange Peel: A Potential Source of Phytochemical Compounds *International Journal of ChemTech Research* ISSN: 0974-4290, 11(02): 240-243.

Nandan M.P., Vangalapati M. (2015). Phytochemical Analysis and Effect of Various Parameters on the Extraction Yield of Flavonoids from the Citrus medica L Peel Extract *International Advanced Research Journal in Science, Engineering and Technology,* 2 (9).

Nair KN, Nayar M P., Hajra, P.K., Nair, V.J., Daniel, P. (1997). Flora of India, *Botanical Survey of India* , 4:229-40.

Nongalleima, K., Ajungla, T., Singh, C.B., Chingakham, C., Singh, B. (2017). Phytochemical, total phenolic, total flavonoid and total flavonol content estimation in Citrus macropteraMontruz. *Journal of Medicinal Plants Studies* 11453:114–118.

Paul S, Das S., Tanvir E.M.,Hossen S Md., Saha M, Afroz R., Islam A Md., Hosaain S Md., Gan H S., Khalil I Md. (2017). Protective effects of ethanolic peel and pulp extracts of *Citrus macroptera* fruit against isoproterenol-induced myocardial infraction in rats. *Biomedicine & Pharmacotherapy* 94:256-264.

Rahman, H., Chinna Eswaraiah, M., Dutta, A.M. (2014). *In-vitro* anti-oxidant activity of Citrus macroptera (varannamensis) fruit peels extracts. *International Journal of Pharmacy and Pharmaceutical Sciences* 6:364–371.

Shukla S and Tyagi B. (2017). Comparative Phytochemical Screening and Analysis of Different Vigna species in Organic Solvents. *Austin J BiotechnolBioeng* 4(3):1084.

Suffredini, I.B., Sader, H.S., Gonçalves, A.G., Reis, A.O., Gales, A.C., Varella, A.D., Younes, R.N. (2004). Screening of antibacterial extracts from plants native to the brazilian amazon rain forest and atlantic forest. *Braz. J. Med. Biol. Res.* 37:379–384.

Syed UJ G. R., S Faridullah, S Sherani, N Jahan., Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from *Ephedra intermedia* Indigenous to Balochistan. *The Scientific World Journal,* 5873648: (7).

Yip R. and. Dallman P. (1988). The roles of inflammation and iron deficiency as causes of anemia, *The American Journal of ClinicalNutrition* 48 (5):295–1300.

An Introduction of Satkara. Retrieved on 7 January duration of immobility in Forced Swim Test reveals 2014. http://gsl.articlealley.com/an-introduction-ofantidepressant activity [16]. satkara-1932990.html