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Pharmacological Activity of *Citrus macroptera*: an overview of a medicinal plant

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Abstract

Plants and plant parts have been extensively used as medicine from the dates of early civilization. They are low in cost and have least side effects. In India, the practice of the traditional medicinal can be traced back to 5000 B.C. *Citrus macroptera* (Mont.) of the family Rutaceae is a bitter fruit bearing plant commonly found in tropical and sub-tropical regions. It is an endangered wild plant of North-eastern India. Various parts of this plant such as leaves, bark, pulp, peel specially fruits have an immense range of medicinal importance and has been used in different kinds of ailments like fever, kidney and liver dysfunction, dyspepsia, cardiovascular disease, stroke and many more to mention. The fruit of this perennial spiny plant has potential antioxidant, cytotoxic, antimicrobial, antihypertensive, antipyretic and appetite stimulant activity. The principal phytochemical constituents of this plant are Ribalinine, Edulinine, Bergamottin, Psoralen, Marmin, Severine, Geipavarine, β-pinene, α-pinene, ρ-cimene, (E)-ocimene, vanilline, Rutin, kaempferol, sabinene, limonene, γ-terpinene and so forth. Furthurmore thrombolytic, antidiabetic, antidepressant, cardioprotective, hepatoprotective activity have been reported on this plant. This review represents a comprehensive study on the various aspects of *Citrus macroptera* with respect to their traditional and medicinal usage or better use as alternative medicine for various kinds of treatments.

Keywords: *Citrus macroptera*, Ethnobotanical pharmacognosic, Phytochemical, Pharmacological

Since time immemorial mankind has been extensively dependent on nature for its survival, livelihood and medicine. Scientists have estimated that approximately 250,000 species of Angiosperms exist on earth, of which a major portion are medicinally important and the rest is yet to be explored for their medicinal beneficiaries. Indigenous people have learned and explored plants those help to cure certain ailments like toothaches, induce labour or cure malaria through their personal experiences and knowledge passed across for generation after generation. But, unfortunately common people unaware of the toxic potential of many plants and thus they might be jeopardizing their health instead benefiting from it(Summer, 2000).There are many aboriginal people belonging to different ethnic groups inhabiting different regions of this world those mainly relies on plants for their basic needs. According to the reports of WHO (World Health Organization), not only the people of tribes but about 80% people of the developing country are dependent upon plant based remedies for several therapeutic purposes (WHO, 2005).

Citrus of the family Rutaceae, is an important vitamin C rich fruit commonly uses as pickles and are taken raw or as desert. It is commonly said "one orange a day, keeps doctor away". Citrus macroptera is a wild relative of cultivated lime (*C.* × *aurantifolia*), lemon (*C. limon*), mandarine *(C. reticulata*), grapefruit(*C. paradisi*) etc., all these plants are potential medicinal plants and are used extensively. *Citrus macroptera* as is a genus of flowering aromatic shrub or tree and have an important role in medicine as well as an economic fruit crop of India. *C.* macroptera is wild

endangered species of north-eastern India (Malik *et al.*, 2013), also known as Melanesian papeda, wild orange (Manner *et al.*, 2013), cabuyao or satkara (Hanelt, 2001). The plant is regarded as a semi-wild species of citrus native to Malesia and Melanesia (Abbott, 2006).

This group of citrus is found growing wild in the forests and adjoining areas of North-East India. There are a wide range of morphological variations which may have arisen due to cross pollination. There is a common practice to group the members of *C. macroptera* into four varieties which are as follows:

C. macroptera var. *macroptera*
C. macroptera var. *annamensis* Tanaka-> *C. combara* Raf.
C. macroptera var. *combara* (Raf.) Tanaka-> *C. combara* Raf.
C. macroptera var. kerrii Swingle -> C. kerrii (Swingle) Tanaka (https://en.wikipedia.org/wiki/Citrus_macroptera.)

The phytoconstituents of *C. macroptera* shows active medicinal properties (Paul *et al.*, 2016). The fruit is rich in phenols, flavonoids, terpenoids, tannins, alkaloids, vit.-C, vit.-E and different beneficial phytocompounds which are very helpful to terminate the chain reaction in human body system produces by the activity of ROS (Reactive Oxygen Species), one of the major reason which lead to the DNA damage. The lupeol and stigmatosterol present in the stem bark is used against ringworms and epilepsy like symptoms. The fruit of *C. macroptera* has potential role as antioxidant. The fruit juice is cytotoxic, antimicrobial, antihypertensive, antipyretic, and appetite stimulant activity. The fruit is also very potential against fever, kidney and liver dysfunction, dyspepsia, cardiovascular disease and stroke (Paul *et al.*, 2016). The pulp and peel of fruit also shows beneficial activity against various ailments. This review has been made on the documented reports available on *Citrus macroptera*.

The Family Rutaceae

Rutaceae, also known as rue or citrus family is usually placed under the order Sapindales comprising about 162 genera and 1650 species. But, several taxonomists have placed this family in different ways which are depicted in table1. The members are distributed throughout the world especially in warm temperate and tropical regions (Chowdhury *et al.*, 2016). The largest number of genera belonging to this family is found in Australia, Africa and often in semi-arid woodlands. The family, Rutaceae includes woody shrubs, trees and a few herbaceous perennials. The species of this family have characteristic strong smell.

In folk medicine this family has great importance. The fruits of *Aegle marmelos*, a member of this family are used in the treatment of chronic diarrhoea, dysentery, peptic ulcers (Rahman and Parvin, 2014) and as a laxative and to recuperate from respiratory infections. Murraya koenigii, another member of this family is rich in alkaloids, mahanimbin, girinimbin, numerous carbazole alkaloids, cinnamaldehyde and it is very good for diabetic patients (Ramsewak, 1999).

Placement of This Family

The placement of this family is shown below in (Table1).

Table 1. Placement of the Family Rutaceae.

	B & H	Cronquist	Takhtajan	Dahlgren	Thorne	APGII/APweb
Division		Magnoliophyta	Magnoliophyta			
Class	Dicotyledons	Magnoliopsida	Magnoliopsida	Magnoliopsida	Angiospermae	
Sub class	Polypetalae	Rosidae	Rosidae	Magniliidae	Rosidae	
Series+/Superorder	Disciflorae+		Rutanae	Rutanae	Rutanae	EurosidsII*
Order	Geraniales	Sapindales	Rutales	Rutales	Rutales	Sapindales

The Genus *Citrus* (L.)

All Citrus have 2n = 18 but some cultivated forms have 27 and 36, but there are no known wild polyploids (Aktar and Foyzun, 2017). Hybrids are readily raised and as because seed can be set through apomixes i.e. without fertilization with embryos arising from the diploid nucellar tissue, appealing forms can readily be propagated. Antoni van Leeuwenhoek showed that such tissue often have several embryos, the first time the phenomenon of polyembryony in any plant was described (Swingle and Reece, http://idtools.org/id/citrus/citrusid/factsheet.php.name, 1967) This is particularly common in the makrut lime, *Citrus macroptera* and the calamondin, *Citrus microcarpa* Bunge. Hybridization and apomixis have led to the taxonomic depredation in the genus. Almost all *citrus* plants diverged from a common ancestor about 15 million years ago, which was about when it diverged from the closely related severinia, e.g. the Chinese box orange. Inexact millions of years ago, the citrus plants diverged into 2 groups i.e. the main citrus genus and the ancestors of the Trifoliate orange i.e. poncirus. It is closely related that it can still be hybridized with all other citrus. These evaluation are made by using genetic mapping of plant chloroplasts. DNA study was published in Nature (2018), depicts that citrus trees originated in the foothills of the Himalayas, in the area of Assam (India), western Yunnan (China), and northern Myanmar.

Core Species and Hybrids of Citrus

Till date most commercial varieties have descended from one or more of the 'core species' i.e. citron, mandarin and pumelo. The core species of citrus, have given rise to a wide variety of hybrids for which the naming is particularly inconsistent. The same local common names may be given to different citrus species and hybrids i.e. citrus with green fruit is called 'limes' independent of their origin i.e. Australian limes, Key limes, wild oranges, sweet limes and wild limes, even they are all genetically distinct. The citrus fruits with similar ancestry may be quite different in name and traits (e.g. grapefruit, common oranges, all citrus hybrids etc). Many traditional citrus groups, such as sweet oranges, limes and lemons seem to be bud sports, families of cultivars that have arisen from distinct spontaneous mutations of a single hybrid ancestry (Fig. 1)

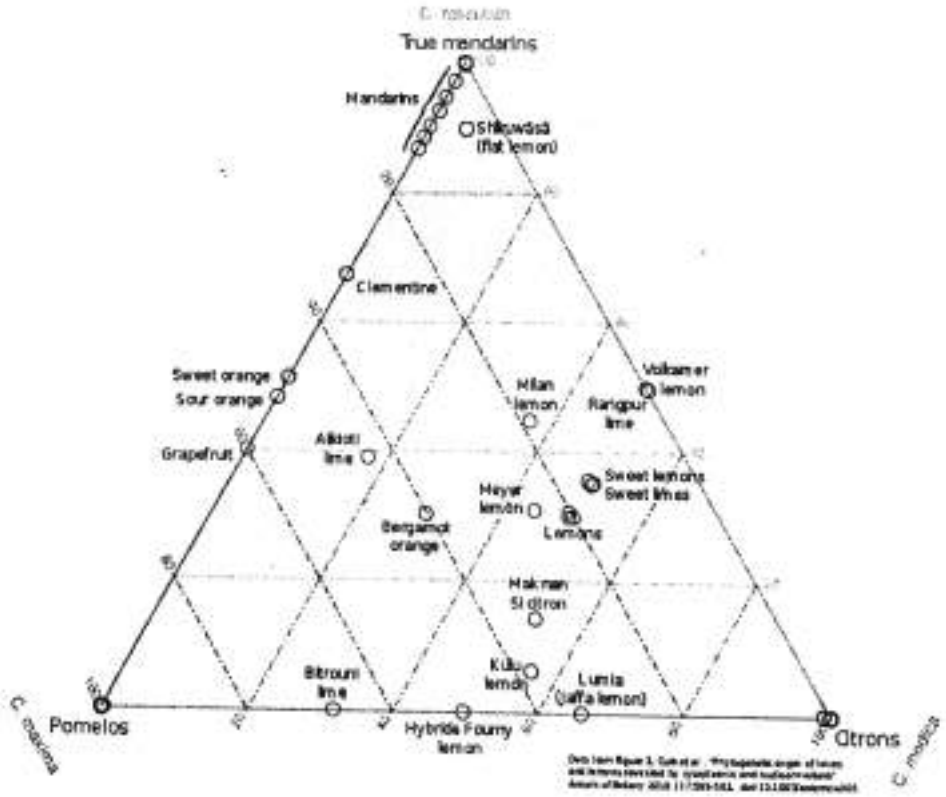


Fig. 1 Hybridization in citrus cultivars
Genetic mixing of three ancestral species

Common Names:

Bengali: Satkara, Hatkora
Guam: *Kabet*
English: wild orange, Melanesian papeda
Tonga: *moli uku*
Samoa: *moli utu*
Fiji: *moli kau*
Vanatu: ghost lime (eng.), *moli* (Ambae, Malo), *mol* (Efate, Pentacost, Santos), *ngoli* (Maewo), *na-moli* (Tongoa). (http://www.guamsustainableag.org/fruittrees/Citrus-citrus%20(11).pdf

Fig. 2: *Citrus macroptera*

Habit and Habitat and Distribution

The plant is found growing in the tropical and subtropical regions. In the subtropics up to 750m (2450ft); in the tropics up to 1600m (5250ft) receiving an annual rainfall ranging in between 900-3000 mm is needed for proper growth of the plant.

The perennial spiny wild orange tree can reach up to 5 m in height with its large "Wings" on petiole. Though the plant is native to Malesia and Melanesia but it is also, widely distributed in India (Assam, Meghalaya, Khasia, Uttar Pradesh), Bangladesh, Myanmar, Java, Thailand, Laos, Vietnam, China (Yunnan, N-Guangxi), American Samoa (Ta'u), Western Samoa (Savaii, Upolu), American Samoa (introduced), Manua Island (introduced), Micronesia (introduced), Yap (introduced), Palau Island (introduced), Sonsorol (introduced), Southern Marianas (introduced), Rota (introduced), Guam (introduced)), Tuamotu Arch. (introduced), Makatea Island (introduced), Nicobar Island (Central Nicobar Island), Sri Lanka, Sulawesi, New Caledonia (introduced), Fiji (introduced), New Guinea, Philippines (Bohol, Mindanao, Cebu), Peninsular Malaysia (northern half of the Peninsula), Sri Lanka (introduced), Society Island (introduced), Tahiti (introduced), Raiatea (introduced)), Trinidad & Tobago (introduced) (Catalogue of Life: Citrus macroptera var. kerrii Swingle)

Morphology of Wild Orange

Leaves: Evergreen leaves are approximate 10-12 inches long (including the petiole) and 2 inches wide, broadly ovate-lanceolate, axillary spines straight, erectopatent about 1/2 inch long.

Petiole: Winged, 2 to 3.5 inches long and from 1-2 inches broad at the top, broad shallow crenular margin, the lenticular glands are very numerous on the midrib, the lamina freely disarticulating from the petiole.

Flower: Whitish, Bracteate, calyx with 4-5 sepals, corolla with 4-5 petals with valvate aestivation, petals elongate, concave, stamens 20 with sub hemispherical, style thick, round, stigma depressed.

Fruit: About 6-7 cm (2.4-2.8 inch), subglobose, somewhat contracted at base, rounded apex.

Seed: Semi-deltoid shape with wrinkled surface and yellow in colour

Peel colour and Other: Pale dull yellow, fairly smooth, moderately thick pericarp.

Pulp colour: Greenish, yellow, dry.

Juice: Acidic and bitter (Chowdhury *et al.*, 2008).

Wild Orange Effectiveness

The effectiveness of C. macroptera is variable. the efficacy of the phytochemicals are most effective in cases like Brain Stroke, Heart Stroke, Heart Disease, Kidney Disease, Liver Disease and are effective in ailments like Cuts, Sore Throat, Insect Bites etc.

Taxonomic Classification

Kingdom: Plantae
Phyllum: Trachaeophyta
Class: Magnoliopsid
Order: Sapindales
Family: Rutaceae
Genus: Citrus
Subgenus: Papeda
Species: macroptera (Aktar et al., 2017)

Traditional and Industrial Uses

Citrus macroptera is very important in industry and traditional form of herbal medicine from very early days of civilization, almost the entire plant is used for medicinal purpose. The decoction of leaves, bark, pulp and the juice of fruit is used for ailments from microbial, bacterial and fungal infections. The fruit juice is antioxidant, antihypertensive, antipyretic, has got appetite stimulant potential and its pulp is effective against different types of chronic diseases. Besides this, the volatile oil is used as perfume and the fruit is an ingredient of pickles, food additives and in confectionary industry etc. The detailed account of usefulness of the plant is depicted in Table 2.

Table 2. Detailed Account of Usefulness of *Citrus macroptera*.

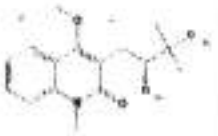
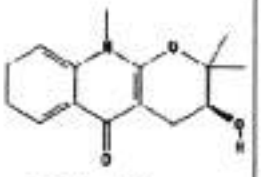
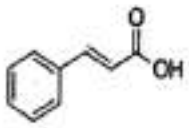
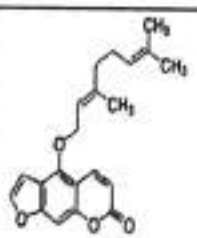
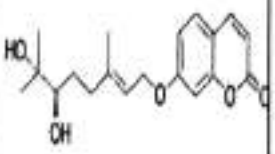
Parts used	Use	References
Leaves	The essential oil from the leaves has anti-microbial, anti-bacterial, anti-fungal activity. Macerated leaves and fruit juice form lather when rubbed and are very useful for washing the hair to free it from lime, which is very much used by the natives to bleach the hair.	(Waikedre *et al.*, 2010)
Fruits	The Wild Orange fruit has significant antioxidant, cytotoxic, antimicrobial, antihypertensive, antipyretic, and appetite stimulant potential. It is very helpful against fever, kidney and liver dysfunction, dyspepsia, cardiovascular disease and stroke. Significant hypoglycemic and neuropharmacological effects are also reported.	(Paul *et al.*, 2015)
	The fruit is extensively used in culinary purposes like cooking, and for pickle preparation. One delicacy of Garo tribes of Meghalaya with this fruit is called as "Wak Chambal Phura".	(Upadhaya, 2015)
	Fruit juice is very good as stomach ailment and flushing stone from kidney.	(Uddin *et al.*, 2014)
Pulp	Effective against different types of chronic disease like Cirrhosis, Diabetes, Myocardial infraction.	(Paul *et al.*, 2017)
Rind	The thick rind is cut into small pieces and cooked either in green and yellow condition in beef, mutton, fish curries, as well as in stews.	(Uddin *et al.*, 2014)
Stem bark	Remedies against ringworms, sickness, and epilepsy like symptoms.	(Desrivot, 2007)
Oil	Used in perfume industry.	(https://en.wikipedia.org wiki/citrus_macroptera)

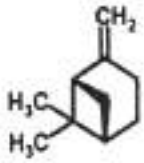
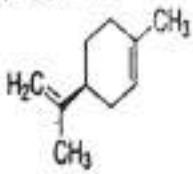
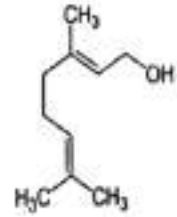
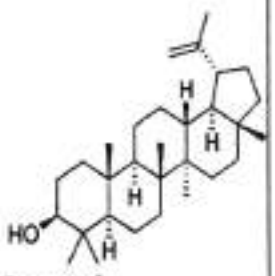
Screening of Phytoconstituents

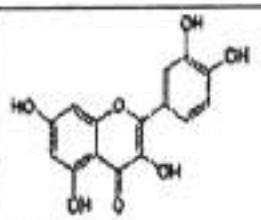
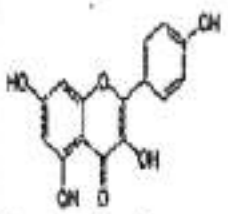
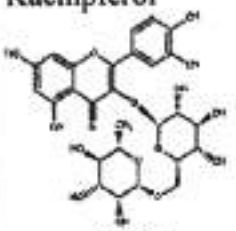
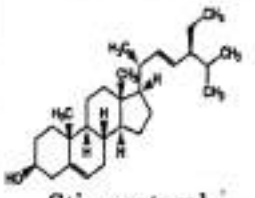
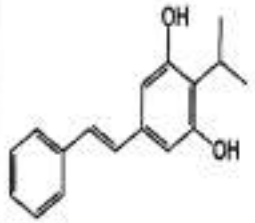
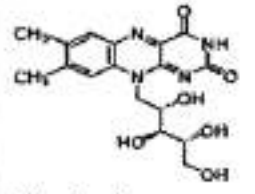
Citrus, an economically and medicinally important genus is used in the treatment of various kinds of diseases and is also extensively used in folk medicine [Lv *et al.*, 2015]. The intake of the Citrus fruit is associated with approximately 10% reduction in odds of developing breast cancer, which makes it a very potent anticancer agent (Cirmi *et al.*, 2017) .The structural polysaccharide Pectin which is very useful to prevent colon cancer, prostate cancer, diabetes, gastroesophageal reflux disease (www.webmd.com/vitamins-supplements) is found in maximum levels in limes, lemons as well as oranges and grapefruits (Healthy eating.sfgate.com/pectin safe vegetarians-9421.html)

The diverse chemical constituents of *Citrus macroptera* have been reported to be used in various kinds of treatments. The characteristic levels of phenol, flavonoids, tannins, alkaloids, terpenoids and also other bioactive compounds has attributed to its antioxidant, antimicrobial, cardio protective, anti-diabetic, anti-inflammation activity (Nongalleima et al., 2017) The various chemical constituents reported in wild orange are listed in Table 3.

Table 3. Various Chemical Constituents of Wild Orange.

Compound Group	Reported compounds	Some Important Structures	Parts in which they found	Effectiveness of the compound group	References
Alkaloids	Ribalinine, Edulinine, Isoplatydesmine, Quinolone.	 Edulinine  Ribalinine	Stem bark	Antifungal, antigenotoxic, antitumor, cytotoxic etc.	(Patel *et al*., 2012
Aromatic compound	Cinnamic acid, Syvingaldehyde, Vanilline, Methyl vanillate	 Cinnamic acid	Stem bark	Used as insecticides, plasticizers, cosmetic industry, antimicrobial agent and so on.	(https://www.scribd.com/doc/350496/The-Uses-of-Aromatic-Compounds, Gaillard *et a*. 1995)
Coumarins	Bergamottin, Psoralen, Marmin, Severine, Geipavarine	 Bergamottin  Marmin	Whole dried fruit	It has characteristic physiological, bacteriostatic, anti-tumour, anti-carcinogenic activity.	(Jain and Joshi, 2012)

Terpenoids	Monoterpenes: β-pinene, α-pinene, ρ-cimene, (E)-ocimene, sabinene, limonene, γ-terpinene, α-thujene, camphene, α-phellandrene, 1,8-cineole, linalool, (E)-tageton, citronellal, isopulegol, borneol, myrtenol, citronellol, geraniol, bornyl acetate, carvacrol, citronellyl acetate, neryl acetate. Oxygenated sesquiterpene: spathulenol, caryophyllene oxide. Other sesquiterpene: β-caryophyllene, α-cardinol, viridiflorol, α-muurolol, longicyclene, α-santalene, (Z)-caryophyllene, (E,E)-α-farnesene, γ-cadinene, humulene epoxide-π, cubenol. Triterpene: Lupeol present in stem bark(Chowdhury et al, 2007).	 β-pinene  Limonene  Geraniol  Lupeol	Seed, Essential oil of Leaves.	These compounds are very active against bacteria, fungi, viruses, protozoa and so on.	(Rana and Blazquez 2012)

Flavonoids	Quercetin, Rutin, Kaempferol, Hespiridin and Neohespiridin	 Quercetin  Kaempferol  Rutin	Rind of fruit, mature and immature fruit.	These polyphenolic compounds possess antioxidant, radical scavenger, antileukemic, vasodilator activity. Also very beneficial for improving blood circulation in brain, in Alzheimer disease and also has potential anti-cancer, anti-ageing and antibacterial activity.	(Sharma, 2006)
Steroid	Stigmasterol	 Stigmasterol	Bark	Potent against antiinflammation, immuno-modulation and many other biologically important activity.	(Ericson, 2014)
Miscellaneous	phenol,vit-C, vit-E, protein, β-carotene, thiamine, riboflavin, ascorbic acid, calcium, phosphorus, magnesium, iron, sodium, potassium, copper, zinc, saponin, stilbene, tannins	 Stillbene  Riboflavin			(Islam *et al.*, 2015)

GC-MS analysis of Citrus macroptera

GC/MS Analysis of the *C. macroptera* essential oil allowed the identification of 35 compounds (Table 4), accounting for 99.1% of the total composition. The oil was mainly constituted of monoterpenes (96.3%), among which β-pinene (33.3%), α-pinene (25.3%), p-cimene (17.6%), (E)-ocimene (6.7%), sabinene (4.8%), g-terpinene (3.1%), and limonene (2.4%). Moreover, spathulenol (0.6%) and caryophyllene oxide (0.5%) were the major oxygenated sesquiterpenes present in the oil. Other sesquiterpenes such as α-cardinol (0.2%), viridiflorol (0.2%), and a-muurolol (0.1%) were also identified.

The oil was mainly constituted of monoterpenes (96.3%), among which b-pinene (33.3%), a-pinene (25.3%), p-cimene (17.6%), (E)-ocimene (6.7%), sabinene (4.8%), g-terpinene (3.1%), and limonene (2.4%) which has some antimicrobial effects. Moreover, spathulenol (0.6%) and caryophyllene oxide (0.5%) were the major oxygenated sesquiterpenes present in the oil. Other sesquiterpenes such as a-cardinol (0.2%), viridiflorol (0.2%), and a-muurolol (0.1%) were also identified (Fig. 2). The chemical composition of the essential oil of C. macroptera showed some resemblances with that of *C. macroptera* (Table 1), for which 38 compounds were identified (accounting for 89.0% of the total oil). The main constituents of C. macroptera essential oil were also monoterpenes with terpinen-4-ol (14.0%), b-pinene (11.9%), a-terpineol (7.6%), 1,8- cineole (6.4%), citronellol (6.0%), and p-cimene (5.6%) as principal compounds. Previous work has shown that limonene is generally one of the main volatiles detected in essential oils from Citrus species, representing from 65 to 90% of the oil, e.g., 65% in C. limon and 91 – 95% in C. paradisi and C. grandis. However, in the present study, limonene accounted only for 2.4 and 4.7% of the essential oils from C. macroptera. Morover, also the content of b-pinene and apinene (58.6%) in the oil from C. macroptera is not similar to that in oils from other Pacific Citrus species, such as C. junos (Rahman et al., 2015) or C. latifolia (Ramsewak, 1999).

Table 4. Phytochemical evaluation of *Citrus macroptera*.

Sl no.	Compound name	Formula	RT
1.	L-Menthyl chloroformate	$C_{11}H_{19}ClO_2$	8.418
2.	Dodecane	$C_{12}H_{26}$	8.857
3.	1,8-Cineole	$C_{10}H_{18}O$	9.809
4.	1 menthone	$C_{10}H_{18}O$	13.461
5.	Dimethyl ester of dimethyl malonic acid	$C_7H_{12}O_4$	19.066
6.	Alpha-copaen	$C_{15}H_{24}$	19.639
7.	2-Methylaminobenzoic acid methyl ester	$C_9H_{11}NO_2$	20.380
8.	n-Pentadecanal	$C_{15}H_{30}O$	27.738
9.	Tetradecanoic acid	$C_{14}H_{28}O_2$	28.668
10.	Neophytadiene	$C_{20}H_{38}$	30.334
11.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_9O$	30.833
12.	3-Eicosyne	$C_{20}H_{38}$	31.205
13.	Hexadecanoic acid, 2-oxo-, methyl ester	$C_{17}H_{32}O_3$	31.267
14.	Furan, 2-octyl	$C_{10}H_{19}Cl$	31.719
15.	Hexadecanal	$C_{16}H_{32}O$	31.995
16.	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	32.105
17.	3-(4,8,12-Trimethyltridecyl) furan	$C_{20}H_{36}O$	32.175
18.	Pentadecanoic acid	$C_{15}H_{30}O_2$	32.783
19.	Cyclopropanecarboxylic acid, oct-3-en-2-yl ester	$C_{12}H_{20}O_2$	33.117
20.	Hexadecanoic acid, 2-oxo-, methyl ester	$C_{17}H_{32}O_3$	33.293
21.	Hexadecanoic acid, trimethylsilyl ester	$C_{19}H_{40}O_2Si$	34.423
22.	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	35.917
23.	2 n Heptylcyclopentanone	$C_{12}H_{22}O$	36.680
24.	Trimethylsilyl (9e)-9-octadecenoate	$C_{21}H_{42}O_2Si$	37.589

1.	2 Ethylcyclohexanol, bromomethyldimethylsilyl ether	$C_{11}H_{23}BrOSi$	38.697
2.	1-Nonadecene	$C_{19}H_{38}$	38.763
3.	Methyl 10-oxooctadecanoate	$C_{19}H_{36}O_3$	38.818
4.	Cyclohexane, decyl	$C_{16}H_{32}$	39.261
5.	Benzyl(dimethyl)silyl methyl decanedioate	$C_{20}H_{32}O_4Si$	39.366
6.	7-Isopropyl-10-methyl-1,5-dithiaspiro[5.5]undecane	$C_{13}H_{24}S_2$	39.537
7.	1-Henicosanol	$C_{21}H_{44}O$	39.763
8.	1-Glyceryl linoleate	$C_{21}H_{38}O_4$	40.054
9.	Oleoyl chloride	$C_{18}H_{33}ClO$	40.112
10.	1-Hexadecanol, 3,7,11,15-tetramethyl	$C_{20}H_{42}O$	40.346
11.	(1-propylnonyl)cyclohexane	$C_{18}H_{36}$	41.154
12.	proximadiol	$C_{15}H_{28}O_2$	41.508
13.	Acetylenedicarboxylic acid, di-(-)-menthyl-	$C_{24}H_{38}O_4$	42.167
14.	10-Undecenyl laurate	$C_{23}H_{44}O_2$	42.697
15.	Spermidine	$C_7H_{19}N_3$	43.406
16.	Cholest-1-eno[2,1-a]naphthalene, 3',4'-dihydro	$C_{35}H_{52}$	44.434
17.	Pregnane-3,11,20,21-tetrol, cyclic 20,21-[(1,1-dimethylethyl	$C_{25}H_{43}BO_4$	45.945
18.	Stigmast-5-en-3-ol, (3.Beta.)	$C_{29}H_{50}O$	47.248

Pharmacological Activity of *Citrus macroptera*

Antioxidant activity

The antioxidant potential of various extracts if *Citrus macroptera* fruit peels were tested and the result showed that the ethanolic extracts had maximum level of antioxidant activity than n-hexane and chloroform extracts because the presence of various phytoconstituents like phenol, flavonoids, alkaloids, glycosides, tannins, volatile oils and so on. The ethanolic extracts also shows its protective role against ferric reducing antioxidant power assay (FRAP), Hydrogen Peroxide(H_2O_2), Nitric Oxide(NO) induced free radical generating chain reaction due to the presence of various active principles which acts individually or synergistically and lead to protection against oxidative stress. Another study shows that hot methanolic extracts also give good antioxidant potential (IC_{50}:178.96 ig/ml). The methanol and Ethyl acetate extracts of wild orange had been studied to determine antioxidant properties. In case of DPPH, NO, the former shows more potential than the latter. However, in case of lipid peroxidation both extracts were found to be potent (Uddin *et al.*, 2014)

Methanolic extract of leaves were evaluated for antioxidant potential. It exerts its oxidative stress by scavenging hydroxyl radicals and inhibiting lipid peroxidation that causes oxidative damage to liver cancer cell line HepG2 cells due to the presence of flavonol (myricetin) in the leaves (68.4 mg/100 g). Ethanolic extract of leaves were screened for the ferric reducing antioxidant power, ß- carotene bleaching and oxygen radical absorbance capacity assay. The study reported that leaves extracts exhibited FRAP value (781 mM TE/g), ß- carotene bleaching activity (35.67%) and ORAC assay (10.51 mmol TE/g). Supercritical carbon dioxide extraction of leaves was reported for the higher total phenolic content (128.9 mg GAE/g extract) than the solvent extraction and higher DPPH radical scavenging activity with the IC50 of 0.065-0.300 mg/ml.22 Juice of Wild orange was reported to possessed high phenolic (490.47 mg GAE/ 100 ml of juice) and flavonoid, (22.25 mg hesperidine

equivalent/100 ml) content and exhibited good antioxidant activity by DPPH and FRAPS methods. Juice showed the scavenging activity against DPPH radicals to have IC_{50} values of 35 mg/100 ml and FRAP value of 89.0 µmol Fe^{2+} equivalent/ 100 mL of juice.

Anti-inflammatory effect

The phenolic compounds, one of largest group of secondary metabolites of plants primarily include polyphenols, flavonoids and tannins. They have the potent biological activities like anti-inflammatory, anti-allergic, antioxidant. One of the causes of inflammation is denaturation of protein. *C. macroptera* inhibit the denaturation of proteins. Three extracts namely aqueous, methanol, aqueous methanol are considered for evaluation of anti-inflammatory activity like protease inhibition assay, albumin denaturation and heat induced haemolysis. Among the three extracts the methanolic extracts shows the much more anti-inflammatory activity than the aqueous and aqueous methanolic extracts with protease inhibitory assay 59.66±0.00 ig/ml, albumin denaturation 104.75±0.00 ig/ml and heat induced haemolysis 100.36±0.008ig/ml (Nongalleima, 2017)

Essential oil from *C. macroptera* reported to exhibit anti-inflammatory activity against acne using 5-lipoxigenase inhibition assay. The major component such as d-limonene in the essential oil could contribute to the inhibitory activity and observed IC_{50} value of 0.05 µl/ml were compared to that of positive control nor-dihydroguaretic acid. D-limonene could inhibit the acne and reduce inflammation as well as reduce the post-acne scar formation and help to relieve acne blemishes.

Methanolic extract of leaves tested to measure the anti-inflammatory activity by 12-O-tetradecanoyl-phorbol 13-acetate (TPA) induces edema formation on ICR mouse ears. Two glycerolipids (1,2-di-O-alinolenoyl-3-O-ß-galactopyranosyl-sn-glycerol (DLGG), 1-O- alinllenoyl-2-O-palmitoyl-3-O- ß-galactopyranosyl- sn-glycerol (LPGG) exhibited higher activity (32 and 43%) than positive control indo-methacin (19%).

Thrombolytic activity

The methanolic extracts of fruits of wild orange shows about 48.87% lysis of the blood clot in thrombolytic activity test as compared to its positive control (streptokinase) which shows 75.18% lysis and negative control 15.82% lysis (Karim *et al.*, 2015).

***In-vitro* cytotoxicity assay**

The brine shrimp bioassay was practical and safe method for the determination of bioactivities of plant products. Brine shrimp criteria of plant extracts were established as LC50 values above 1000ig/ml were non-toxic, between 500-1000 ig/ml were weakly toxic and toxic below 500 ig/ml. Methanol and Ethyl-acetate extracts were evaluated for the assay and they show a very good LC_{50} values of 14.58 ig/ml and 19.13 i/ml which showed toxicity due to the presence of different types of secondary metabolites like terpenoids, steroids, tannins, alkaloids etc (Park *et al.*, 2004).

Antimicrobial activity

The methanol and ethyl acetate extracts of wild orange fruit were tested using the disc diffusion technique against bacterial species where ethyl acetate extract showed more moderate antimicrobial activity than methanolic extract. The ethyl-acetate extracts showed highest zone of inhibition again two Gram positive bacteria i.e. *Bacillus subtilis* (7.25±0.353mm), *Staphyllococcus aureus* (6.75±0.353mm) and one Gram negative bacteria i.e *Escherichia coli* (7.5±0.707mm) as compared to standard Azithromycin against the above pathogens which had inhibition zone 15.5±0.707mm, 27.5±0.707mm, 25.5±0.70mm respectively (Uddin et al. 2014). However, maximum level of inhibitory activity were seen in case of Bacillus with MIC (Minimum Inhibitory Concentration) 1.25mg/ml (Miah *et al.*, 2005)

Wild orange leaf oil exhibited a good activity against *Trichophyton mentagrophytes* with a MIC of 12.51 ig/ml (Waikedre, 2010).

It is an established fact that essential oils from *Citrus sp.*, possessed antimicrobial effect such as antibacterial and antifungal activity. So the essential oils from citrus have used to improve the shelf life and safety of minimally processed fruits, skimmed milk and low-fat milk. The
essential oil and crude ethanol extract of Wild orange peel showed greater antibacterial action against twenty types of Salmonella and five species of enterobacteria than the crude ethanol extract of wild orange leaves. Volatile oil from the Wild orange peel exhibit the antimicrobial activity against *B. subtilis, E. coli, S. typhimurium* and *S. aureus*.

The crude extract from peel could inhibit the growth of Candida albicans which causing dandruff on the scalp. The Wild orange aromatic oil could inhibit the growth of *C. albicans* and having more effective than positive control ketoconasole. The essential oil from the leaf of Wild orange exhibited insecticidal properties against *Spodoptera litura* at 26.78 µL/g.
Supercritical fluid extract of *C. macroptera* (stem and bark) have the possibility to be applied as a constituent of cosmetic products and medicines because they showed highest antibacterial activity against on *Bacillus subtilis*, moderate activity on *B. cereus* and *Staphylococcus epidermidis*, and weak activity against *S. aureus* and *Propionibacterum acnes* which are known to cause various type of skin infections.36 At 5 and 10% concentration the Wild orange extract could inhibit the fungal spore germination of *Colletotrichum gloeosporioides* and *Fusarium* sp.,37 Isolated coumarins benzenoid derivatives and quinolinone alkaloid from the acetone extract of Wild orange root were found to exhibit antibacterial activity against *Acinetobacter baumannii* and *E. coli* with MIC values < 3.132 and 3.12 mg/ml.

Wild orange peel has the ability to act as a natural antimicrobial agent and showed activity against *B. cereus*, *Salmonella typhi* and *Staphylococcus aureus*. The ethyl acetate extract from wild orange peel exhibited broad spectrum of inhibitory activity against Gram positive bacteria, yeast and molds including *Staphylococcus aureus*, *Listeria monocytogenes*, *Saccharomyces cerevisiae* and *Aspergillus fumigatus*. The major component such as limonene, citronellal, sabinene and ß-pinene, caryophyllene may contribute to the antimicrobial activity.

Alcoholic extract of wild orange peel has been exhibited the antibacterial activity against *S. aureus, B. cereus, Vibrio cholera Ogawa* and *V. parahemolyticus*. The methanolic peel extract of *C. macroptera* fruit possess antibacterial activity on human pathogenic bacteria such as *S. aureus, S. typhi, E. coli, K. pneumonia* and *Proteus vulgaris*. The maximum inhibition zone was recorded against *S. aureus* and *S. typhi* an inhibition zone of 19 mm and 22 mm respectively.

Cardioprotective and Hepatoprotective activity

The effect of methanol extract of *Citrus macroptera* fruits was administered at 250, 500, 1000 mg/kg body weight in Sprague-Dawley female rats to evaluate the toxic effect of extract by using some biochemical and hematological parameters. There was no significant effect on body weight, percent water content, relative organ weight and hematological parameters on female rats. The rats also showed significant decrease in the levels of triglycerides, cholesterol, low density lipoprotein and very low density lipoprotein which leads to decrease in cardiac risk ratio, castelli"s risk index 2, atherogenic co-efficient, atherogenic index of plasma at all doses. 500mg/kg does decreased alkaline phosphatase ($p<0.05$). The complete absence of inflammatory and necrotic features in primary body organ was confirmed by histopatholigical study, justified the cardioprotective, moderate hepatoprotective activities of fruit extracts (Uddin *et al.*, 2014)

The ethanol extract of wild orange peel and pulp against isoproterenol (IPO)-induced myocardial infarction (MI) of Male albino Wister rats was performed to evaluate its cardioprotective role. Pre-treated rats with C. macroptera peel and pulp extracts prior to the administration of Ipo showed

significant improvement in many of the investigated biochemical parameters like cardiac troponin I, cardiac marker enzymes, lipid profile and oxidative stress marker. However, the fruit peel extract showed potential cardioprotective activity than the fruit pulp extracts (Paul *et al.*, 2017).

Anti diabetic activity

The methanol extract of fruit at a dose of 250 mg/kg showed significant decrease in the level of glycated hemoglobin ($p<0.05$) that leads to its antidiabetic activity (Uddin *et al.*, 2014)

The C. macroptera fruit extract also showed its anti-diabetic activity possibly by reducing blood glucose and serum insulin level which had been shown to alleviate hyperglycemia-associated oxidative stress in experimental type-2 diabetic rats[50]. The hypoglycemic effects assumed to occur via steroid, terpenoid and saponins identified in wild orange fruits. á-amylase which causes breakdown of starch into simple sugars and increase glocose level in blood, the extract of fruit also revealed moderate á-amylase inhibitary activity (IC_{50} value =3.638±0.190mg/dl) as compared to acarbose, thus reducing the blood sugar level which is quite beneficial for human beings.

Neuropharmacological activities

The ethanolic extract of C. macroptera fruit peel showed significant neuropharmacological effect against oxidative stress by increasing the levels of cellular antioxidant enzymes like superoxide dismutase, catalase in rats. The phytoconstituents like polyphenol, flavonoids, organosulphur compounds had also been reported to contribute the neuropharmacological activity.

Acute oral toxicity of ethanolic extracts of C. macroptera fruit peels were carried out and it was found to be non-toxic. The anxiolytic and antidepressant activities were found in ethanolic extract of C. macroptera fruit peels in experimented mice models. However the antidepressant activity was also found to increase the power of brain antioxidant enzyme level in experimental animal model (Chowdhury, 2008)

Anti tumor activity

Methonol extract of leaves were evaluated for hepatocarcinogenic activity against 2-amino-3,8-dimethylimidazo quinoxaline. Wild orange exerts strong promotive potential on 2-amino-3, 8-dimethylimidazo quinoxaline induced hepatocarcinogenesis in the rat model. The presence of some active chemicals in the leaves of *C. macroptera* could significantly enhance the hepatocarcinogenic effect.

Two glycerolipids (1, 2-di-O-a-linolenoyl-3-O-ß-galactopyranosyl-snglycerol, 1-O-a linllenoyl-2-O-palmitoyl-3-O- ß- galactopyranosyl- sn-glycerol)from methanol extract of leaves of *C. macroptera* were evaluated to inhibit the tumour promoting activity of 12-O-tetradecanoyl-phorbol 13-acetate in mouse skin with dimethylbenz (a) anthracene and 12-O-tetradecanoylphorbol 13-acetate. Both lipids were potent inhibitors of tumour promoter induced Epstein-Barr virus activation.

Conclusion

Now-a-days there is an increasing interest among the researchers to discover, design and evaluate the drugs that can be used against various kind of treatments, obtained from natural resources and they are completely safe for human beings. Thorough screening of literature available on *Citrus macroptera* shows that it can be used against various types of ailments. This wild orange plant has inimitable source of variety of compounds and plethora of medicinal activities. The different parts of this plant have been screened for some pharmacological activities and found to possess antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, cardioprotective, neuropharmacological activities etc. In future, the phytoconstituents and pure compounds need to be evaluated with proper scientific manner using experimental animal models and clinical trials to explore their full therapeutic activity.

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