Indian J. Applied & Pure Bio. Special Vol. 2(1): 236-240 (2021). **ISSN: 0970-2091**
A web of Science Journal
National e-Conference on Biodiversity Building and Natural Resource Management (NC BBNRM) Date: 22nd-23rd, December, 2021

Antioxidant and Gcms Analysis of Lesser Known Species of Citrus

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Abstract

There is always an growing demand of natural remedies from natural sources which can be a replacement of the synthetic therapeutic drugs and lessen their side effects. The aim of the present study was to investigate the antioxidant potential and the active phytocomponents of Citrus unshiu which might be responsible for its antioxidant effect. At higher concentration (200µg/ml) the leaf extract of C. unshiu showed 76.58% of inhibition. After GC-MS analysis total twenty phytocompounds were detected most of which showed antioxidant, antiinflammatory, antithyroid activity and so on. Overall, the present study suggested that the crude leaf extract of C. unshiu could be used as a promising antioxidant and its phytocompounds has several beneficial effects.

Medicinal plants are considered a repository of various types of bioactive compounds possessing different therapeutic properties. The vast array of therapeutic effects associated with medicinal plants includes antiinflammatory, antiviral, antitumor, antimalarial, and analgesic activity[8]. The medications came from herbal sources are generally regarded as safe at the concoction dosage, based on their historical usage in

various cultures. Besides plants are often regarded as alternative sources of medicines and are invaluable in the ethnomedical treatment of diverse ailments[11].

Citrus plant and its other portions are a significant part of the human diet. Previous investigations have proved that citrus fruits are a good source of phenolics and are rich in numerous biologically active compounds such as vitamin C, phenolic acids, and flavonoids[6]. The leaves of Citrus macroptera has been proven to reduce inflammation in in vivo mice model[5]. *Citrus unshiu*, a plant of Rutaceae family commonly known as satsuma orange, satsuma mandarin, Christmas orange and tangerine has a sweet taste, seedless and easy to peel. *C. unshiu* contains peels that can be use as bioenergy source because the peel contains pectin, hemicellulose, cellulose and lignin as the main cell wall components[4]. Besides it possess good antibacterial activity[12].

However no significant work has been done with *C. unshiu* leaf extract especially so far, there is no report of antioxidant, and GC MS analysis of the extract. Therefore in the present study, antioxidants activity has been done which is directly associated with oxidative stress-related diseases[3]. Particular significance was also given to the active chemical compounds which were recognised in GC-MS analysis which might be accountable for its potent bioactivity.

Collection and identification of plant materials :

The leaves were collected from the NBU Campus, Siliguri. The plant sample was identified by Plant Taxonomy and Biosystematics laboratory in the Department of Botany.

Preparation of extract:

Leaves were washed with running tap water for three times to remove the unwanted dirt. After soaking, leaves were crushed in grinder. After-that the extraction was performed in Soxhlet apparatus (plant material: solvent was 1:10 m/v) for 7–8 hour using methanol as solvent. The leaf extract was then concentrated under reduced vacuum pressure at 45 °C in a rotary vacuum evaporator (Buchi Rotavapor R-3, Switzerland). The concentrated extract thus obtaines was then stored in – 20 °C for further use.

In vitro antioxidant assays:

Determination of hydrogen donating ability (DPPH assay):

The free radical scavenging activity by DPPH assay was performed following the method reported by Saha *et al.*[10] with little modifications in extract concentration. Different concentrations (50–200 µg/ml) of the plant extract were prepared and mixed properly with freshly produced DPPH solution i.e. 1 mM, diluted in 95% methanol and kept in dark or amber bottles. Using Bio-Rad micro plate reader the optical density (OD) was measured at 517 nm and compared with the standard ascorbic acid. The % of inhibition was calculated by using the following Equation:

Percent (%) of scavenging = $A0 - A1 / A0 \times 100$

where A0 = absorbance of the control and A1= absorbance in the presence of samples and standard.

Determination of reducing power :

The Ferric reducing power (FRP) assay was determined by the method of Saha *et al*.[10] with minor alterations in extract doses. Different concentrations (50–200 µg/ml) of the leaf extract of C unshiu (500 µl) were mixed with 0.2M of phosphate buffer (pH 6.6) and 0.1% of potassium hexacyanoferrate, accompanied by incubation at 50 °C for 30 min in a water bath. After incubation, 1ml of TCA (10%) was added to the mixture to inhibit the reaction. The aqueous portion of the upper level of the mixture was then conveyed to another tube and mixed with 2 ml of distilled water followed by 2 ml of 0.01% of $FeCl_3$ solution. The mixture was left for 25 min at room temperature (RT) and the absorbance was measured at 700 nm. BHT was used as standard.

GC-MS analysis:

The biologically active compounds of C. unshiu leaf extract were identified by GC-MS analysis[2]. Thermo Scientific Trace 1300 GC, equipped with ISQ-QD (Thermo Scientific) single quadruple mass analyzer and TG-5MS column were employed for the identification of bioactive compounds. ThermoXcalibur TM software version 2.2 was used to determine all the samples. Automated Mass Spectral Deconvolution and Identification System (AMDIS) version 2.70 was employed for the interpretation of MS data.

Statistical analysis:

All qualitative data have been reported as the mean ± SD of three measurements. Statistical analysis and representation of statistical data were done using the one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test, where $\alpha < 0.001$ was considered as significant.

DPPH Assay:

In the present antioxidant profiling, *C. unshiu* leaf extract exhibited free radical scavenging activity (76.58 ± 0.6 at 200 µg/ml) than the respective standard (ascorbic acid) that showed a value 78.2 ± 0.3 at same concentration (Fig. 1). This was evident from the development of yellow coloration after adding DPPH. In the present study, *C. unshiu* leaf extract showed potential free radical inhibiting activity. DPPH is a free radical and it can accept an electron or hydrogen radical to become stable. After-that it reacts with reducing agent changing the colour of the solution into pale yellow[5]. Thus, DPPH scavenging activity by *C. unshiu* leaf extract proves the presence of significant antioxidant properties.

Ferric reducing power assay:

The reducing power of *C. unshiu* leaf extract was found to be increased in a dose dependent manner *i.e.* at 200 µg/ml it showed the absorbance value 2.14±0.2 as comparable to the reference compound BHT which showed the absorbance value 2.5±0.5. So, the reducing activity of *C. unshiu* leaf extract was not much low as compared to its standard BHT. One of the major cause of neutralizing these free radicals might be the presence of phenol and flavonoid compounds in *C. unshiu* leaf extract[10].

Table-1. GC-MS analysis of *C. unshiu* leaf extract.

Sl No.	Compounds	RT
1.	2-Hydroxy-gamma-butyrolactone	6.354
2.	Furaneol	7.369
3.	Pentanal	7.678
4.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	8.532
5.	Acetic acid, phenyl ester	9.209
6.	5-Hydroxymethylfurfural	9.990
7.	2-Methoxy-4-vinylphenol	10.813
8.	2-Deoxy-D-galactose	16.089
9.	n-Hexadecanoic acid	18.378
10.	9-Octadecenoic acid (Z)-, methyl ester	19.581
11.	Phytol	19.697
12.	1-Nonadecene	20.058
13.	Ethyl Oleate	20.188
14.	Palmitic Acid	23.015
15.	Vitamin E	29.771
16.	1H,3H-Furo[3,4-C]Furan, Tetrahydro	30.021
17.	Cis caryophyllene	31.236
18.	D-limonene	32.754
19.	Beta pinene	32.8
20.	Stigmasterol	33.709

GC-MS analysis :

Total 20 compounds were detected in GC-MS analysis. GC-MS results have depicted in Table-1. Several compounds with potent therapeutic importance have been detected with the help of GC-MS analysis. Several phytocompounds belonging to long chain fatty acid moiety and their derivatives such as hexadecanoic acid, octadecadienoic acid, and phytol have been identified in the extract. Interestingly, these long chain fatty acids play an important role in plant development. Among the various compounds pentanal is used in flavorings, resin chemistry and rubber accelerators. Ethyl oleate has very good antimicrobial effect[1]. Stigmasterol which is a good antioxidant compound also shows hypoglycemic and thyroid inhibiting activity[7]. Vit E is a very good anti inflammatory activity[9]. Most of the compounds we got from GCMS is potent bioactive agents.

Living cells have a tendency of generating reactive oxygen species (ROS) in day to day activities. Continuous production

of ROS shifts cells oxidant-antioxidant pool. Besides, reactive oxygen species are involved in liver damage, kidney dysfunction, aging-related disorders, and inflammation. Juice of Citrus is known for ages as important health drink. There are several work depicting the importance of *C. unshiu* peel extract. However, the activity of leaf extract of *C. unshiu* is largely elusive. In the present study we have shown that *C. unshiu* leaf extract is a potent source of antioxidant as it successfully inhibits the free radicals generated by DPPH and it has great reducing power. Besides, several phytocompounds detected in GCMS analysis which has various activity like antiinflammatory, antimicrobial, antioxidant etc. So, from the present study it could be said that the leaf extract of *C. unshiu* could be a value addition in the list of health product related to Citrus. However, further study is required before its commercialization.

Authors' contributions :

AS conceived the idea. AS, ML and SB designed the experiment. All the authors have contributed in drafting the manuscript and approved it.

Funding:

Not applicable.

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