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MICROWAVE ASSISTED EXTRACTION OF LUPEOL FROM *CRATEAVA NURVALA* BUCH HAM AND IT'S *IN VITRO* ANTIOXIDANT SCREENING

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ABSTRACT
The present work reports on a rapid isolation of a colourless, pentacyclic triterpene lupeol from *Crateava nurvala* Buch Ham by the use of newly developed microwave assisted extraction. Several extraction parameters such as temperature, time, and amount of solvent were optimized first. The conventional heating for 12 h provided 0.616 % lupeol and by microwave heating at 210 W for 20 min, the yield was 0.718 %. The results suggested that the proposed microwave method was effective, alternative and superior to soxhlet extraction for the extraction of lupeol. The isolated compounds were found to be same by UV, HPTLC, and ^{1}H-NMR studies. The isolated lupeol was tested for in vitro antioxidant activity, and showed a potent free radical scavenging activity.

Key Words: *Crateava nurvala*, Microwave Assisted Extraction**,** Lupeol, Triterpene, Antioxidant activity.

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INTRODUCTION

Lupeol, a pentacyclic triterpene, isolated from *Crataeva nurvala* Buch Ham [1]. Pentacyclic triterpenes have a wide spectrum of biological activities and some of them may be useful even in medicines. These include the pentacyclic lupane-type triterpenes which are represented by a diverse assemblage of bioactive natural products. Among this class of compounds, lupeol [lup- 20(29)-en-3b-ol] occurs across a multitude of taxonomically diverse genera [2]. It is commonly found in plants viz. *Hieracium pilosella* [3], *Tamarindus indica* [4],

Crataeva nurvala (Buch Ham) [5], *Arbutus unedo* [6], *Betula platyphylla* [7], latex of *Leptadenia* hastate [8], roots of *Anemone raddeana* [9], bark of *Gossampinus malabarica* [10] and *Acacia mellifera* [11], etc.. Stem bark of *C. nurvala*, Buch Ham (Capparidaceae), is of particular interest as it is endowed with an excellent yield of lupeol. Among the compounds isolated from the stem bark, lupeol was identified as a major component in association with α, and β-amyrin.

Lupeol exhibits chemoprevention, anti-urolithiatic [12], anti-cytotoxic [13], anti-inflammatory [14], glycolate-induced experimental lithiasis in rats [15], cytoprotective, hepatoprotective [16], antiarthritic [17], cytotoxicity against human hepatocellular carcinoma (Hep-G2) and human epidermoid carcinoma (A-431) [18]. Cardioprotective effects in hypercholesterolemic condition [19], inhibit cytokine production (TNF-α and IL-1β) in lipopolysaccharide-treated macrophages [20], lowers serum lipids and improves antioxidant status in hypercholesterolemic animals [21], lupeol and its ester derivative reduces the oxidative and inflammatory stresses induced on hypercholesterolemia conditions. It inhibits cancer growth *in vitro* and *in vivo* and ameliorates inefficiency of cancer cells to undergo apoptosis [22]. Lupeol administration induced a remarkable decrease in kidney oxalate level and also was effective in counteracting the free radical toxicity by bringing about a significant decrease in peroxidase level and increase in antioxidant status [23].

Recently the use of microwave energy for extraction of constituents from plant material has proved to be tremendous in the research interest. Microwave-assisted extraction (MAE), also called microwave extraction, is a new extraction technique, which combines microwave and traditional solvent extraction. The Soxhlet extraction process is time consuming, laborious and makes use of bulk amount of organic solvents. As the heating process continues for long hours, the approach possibly involves high risk of thermal decomposition of target molecules. The technology for microwave-assisted chemistry has matured significantly since the pioneering work on the use of microwave energy to accelerate solvent extraction procedures for analytical sample preparation [24]. MAE has been employed for the extraction of pollutants such as polynuclear aromatic hydrocarbons [25], phenols in soil samples [26], and natural products such as silymarin from milk thistle seeds [27], quercetin from *Psidium guajava* leaves [28], and ginsenosides from ginseng root [29] etc.

In the conventional procedure the stem bark of *Crataeva nurvala* was extracted with petroleum ether and concentrated to obtain lupeol. Since the petroleum ether is microwave transparent, this procedure without modification can not be used for the microwave extraction of lupeol. A mixture of petroleum ether and water was used for microwave extraction of lupeol with an improved yield.

MATERIALS AND METHODS

Equipments

Catalyst Scientific Microwave Oven with variable power output ranging between 140 W and 700 W; Conventional Soxhlet equipment and Buchi Rotovapor. Catalyst Scientific Microwave-(The extraction system consist of a microwave extractor manufactured by Catalyst Systems (Pune, India) equipped with a magnetron of 2450MHz, a reflux unit, 10 power levels (140W to 700W), time controller, exhaust system, beam reflector and a stirring device. The whole system was opened and run at atmospheric pressure. Closed vessel microwave extraction allows extraction solvents to be heated rapidly to temperature two or threefold higher than their atmospheric boiling points, which results in shorter extraction times. Stirring is possible and it makes the extraction conditions more homogenous); Conventional Soxhlet equipment; and Buchi Rotovapor.

Conventional extraction technique

Air dried stem bark of *Crateava nurvala* (10 g) was extracted with petroleum ether 50 ml in a Soxhlet apparatus for 12 h [30]. The petroleum ether extract was then concentrated. The residue obtained was then crystallized by the solvent acetone: ethanol (50:50) to obtain lupeol. The product was then completely dried, weighed and its chromatographical and spectral studies were carried out.

Microwave assisted extraction

Air dried stem bark of *Crateava nurvala* (10 g) were also extracted with 50 ml petroleum ether and 20 ml distilled water using microwave power at 210 W intensity for 40 min. When the irradiation period was complete, samples were removed from the microwave cavity and allowed to cool to room temperature. The petroleum ether extract was then concentrated. The residue obtained was then crystallized by the solvent acetone: ethanol (50:50) to obtain lupeol. The product was then completely dried, weighed and its chromatographical and spectral studies were carried out.

In Vitro antioxidant activity

The isolated lopeol was tested for *in vitro* antioxidant activity using 2 standard methods. The concentrations of the sample and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625, 7.812, upto 0.025 μg/ml. The absorbance was measured spectrophotometrically against the corresponding blank solution. The percentage inhibition was calculated by using the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD control - OD sample}}{\text{OD control}} \times 100$$

IC_{50}, which is the concentration of the sample required to scavenge 50% of free radicals was calculated.

DPPH assay

The assay was carried out in a 96 well microtitre plate. To 200 μl of DPPH (2, 2' diphenyl-1-picryl hydrazyl) solution, 10 μl of each of the sample or standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37 ^{o}C for 30 min and the absorbance of each solution was measured at 490 nm using ELISA reader [31].

Scavenging of ABTS [2, 2'-azino-bis (3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt] radical cat-ion assay

Accurately weighed 54.8 mg of ABTS and dissolved in 50 ml of distilled water (2 mM) and potassium per sulpha (17 mM, 0.3 ml) was added. The reaction mixture was left to stand at room temperature overnight in dark before usage. To 0.2 ml of various concentrations of the sample or standards, added 1.0 ml of distilled DMSO (Dimethyl sulfoxide) and 0.16 ml of ABTS solution to make the final volume of 1.36 ml. Absorbance was measured after 20 min at 734 nm [32].

RESULTS AND DISCUSSION

Study shows that microwave- assisted extraction has many advantages, such as shorter time, less solvent, higher extraction rate, better products with lower cost. Soxhlet method usually needs a few hours, even more than 20 h, while microwave-assisted extraction only needs a few minutes [33, 34]. The use of microwave energy enables fast dissolution, drying, acidic digestion and extraction of organic compounds from complex environmental matrices; its main advantages are reduced solvent volume and time consumption. The conventional Soxhlet extraction of lupeol requires more time; to reduce its duration of extraction, microwave assisted extraction was carried out.

The conventional yield of lupeol involving 12 h heating was found to be 0.616 %. Using petroleum ether and water mixture (50 + 20ml), a simple microwave extraction procedure was developed. The product was obtained when 140 W to 245 W intensity was used. However the yields higher than the conventional method were obtained only at 210 W intensity. The best results of 0.718 %. were obtained at 210 W intensity and heating for a period of 20 min, thus saving 11 h 40 min and with a 16.5 % increased yield.

The UV spectrum of standard solution of lupeol obtained by conventional procedure showed λmax at 276, 273, 239, 229 nm and by microwave method the same solution showed λmax of 278, 274, 238, 230 nm respectively. The HPTLC separation of lupeol for both conventional and microwave method yields a single spot with R_f value 0.21 and 0.23 under the same conditions. ^{1}H NMR of conventional isolated lupeol shows δ4.70 (s, 1H, H-29), δ4.56 (s, 1H, H-27), δ3.18(d, 1H, 3-OH), 2.39(m, 1H, 19-H), δ1.91 (m, 1H, H-21), δ1.69 (s, 3H, H-30), δ1.53 (d, 1H, H-11), δ1.29 (q, 1H, H-12), δ1.04 (s, 1H, H-23), δ0.97 (s, 3H, 27-H), δ0.91 (t, 1H, H-18), δ0.81 (s, 3H, H-25), δ0.78(s, 3H, H-28), δ0.69 (d, 1H, H-5).The microwave isolated lupeol shows δ4.70 (s, 1H, H-29), δ4.56 (s, 1H, H-27), δ3.19(d, 1H, 3-OH), 2.40(m, 1H, 19-H), δ1.92 (m, 1H, H-21), δ1.69 (s, 3H, H-30), δ1.53 (d, 1H, H-11), δ1.29 (q, 1H, H-12), δ1.04 (s,

1H, H-23), δ0.97 (s, 3H, 27-H), δ0.92 (t, 1H, H-18), δ0.81 (s, 3H, H-25), δ0.78(s, 3H, H-28), δ0.69 (d, 1H, H-5).

Invitro antioxidant methods were performed, the lupeol showed potent antioxidant activity in DPPH and ABTS, since IC50 values were found to be less than the respective standards. The results are shown in the table1.

Table-1 *In vitro* antioxidant activity of the isolated Lupeol

Sample/Standards	IC_{50} values ± SE (µg/ml) by methods*	
	DPPH	**ABTS**
Sample: Lupeol	2.30± 0.18	0.99± 0.05
Standards		
Ascorbic acid	11.25 ± 0.49	2.69 ± 0.05
Rutin	0.51 ± 0.01	3.91 ± 0.10

*Average of three determinations.

CONCLUSION

Ways to minimize the consumption of energy and developing efficient isolation and purification processes is of high importance now. The present work demonstrates the feasibility of MAE for isolation of lupeol. In conclusion, we have successfully developed simple, fast, economic and eco-friendly method for isolation of lupeol from *Crateava nurvala.* The present work is a good contribution towards providing green alternative procedures. MAE offers the added advantages of low consumption of solvent, a short extraction time, low energy consumption and excellent reproducibility.

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