

IDENTIFICATION OF PHYTO-CHEMICALS OF *SYZYGIUM CUMINI* FRUIT

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ABSTRACT

This study was carried out to identify phyto-chemicals of *Syzygium cumini* (L.) Skeels. For this, dry and powdered fruit of the tree was extracted in methanol and partitioned with *n*-hexane and chloroform. Using solvent system of chloroform: *n*-hexane (30:70), four compounds were identified on a TLC plate which were separated on a preparative TLC and further purified on HPLC followed by identification through GC-MS analysis. These compounds included ethyl iso-allocholate (1), oxime-, methoxy-phenyl (2), heptacosane (3) and octadecane (4). A thorough literature survey was conducted to search for various bioactivities of the identified compounds. These compounds were found to possess antioxidant, anti-inflammatory, antimicrobial, anticancer, antifungal, antibacterial and/or antiasthma properties.

Key-words: Bioactive constituents, HPLC, Fruit, Phyto-constituents, *Syzygium cumini*.

INTRODUCTION

Syzygium cumini (L.) Skeels, family Myrtaceae, is an evergreen plant native to South-east Asia, Indian Sub-continent, Australia and tropical America (Sherif, 2019). It is highly unevenly worldwide distributed plant and is very often cultivated to subtropical and tropical regions (Plathia *et al.*, 2018). The plant produces plums that are astringent, mildly sour or sweet in flavor, commonly known as black plum, Indian blackberry, java plum, jamun, purple plum, damson plum and Jamaica (Ali *et al.*, 2016). The fruit is 1.8 to 3.2 cm long, oval shaped with a deep purple color having a hard seed inside (Chaudhary and Mukhopadhyay, 2012). The unripen fruit juice is being used in jelly, squash, vinegar and preservatives preparation (Agarwala *et al.*, 2019). It is rich in fructose, mallic acid, anthocyanins, tannins, gallic acid, glucose, citric acid, raffinose, petunidin, malvidin cyanidin and diglycosides of commercial importance (Singh *et al.*, 2018).

Traditionally, the fruit possesses effective antibacterial, antioxidant and anti-inflammatory properties (Singh *et al.*, 2016). It produces saccharides, polyphenols and proteins which have antidiabetic effects and have been in practice against diabetes for more than 100 years (Amudha *et al.*, 2018). Moreover, antimellin, glycoside, jambosine and alkaloids are also present in the fruit that convert starch into sugar with effective decrease in blood pressure and ellagic acid contents (Suryajayanti *et al.*, 2017). It is also effectively used for the cure of inflammations, ringworm, dysentery, ailments, cough, pimples, piles, stomachache, diarrhea, cancer, colic and blisters (Banerjee and Rana, 2020). The aim of this study was to identify various phyto-components in chloroform fraction of methanolic fruit extract of *S. cumini* and to collect information regarding various bioactivities of the identified compounds from literature.

MATERIALS AND METHODS

Fresh fruits of *S. cumini* were collected from Lahore, Pakistan, dried and ground to a coarse powder. Material was extracted in methanol for two weeks and after filtration, the solvent was evaporated on a rotary evaporator. The obtained methanolic extract was mixed in water and partitioned successively using *n*-hexane and chloroform. A number of solvent systems were tried and finally chloroform: *n*-hexane (30:70) was used on TLC plate. To isolate these compounds, silica gel plates (20 × 20 cm²) were used in preparative thin layer chromatography (PTLC). With the help of a fine needle, the four compounds were scratched from surface of the plate. To detach the compounds from silica gel, they were dissolved in chloroform: methanol (50:50). After settle down of the silica gel, the solvent mixture with dissolved compounds was separated and evaporated at 30 °C to get the compounds. For further purification of the isolated compounds, HPLC was used. HiQ Sil C18, 4.6 × 250 mm, 5 micron column was used in HPLC. A volume 20 µL of each sample was used with 20 min run time and 0.7 mL min⁻¹ flow rate. HPLC chromatograms of the four sub-fractions of chloroform fraction are shown in Fig. 1. Detection was made at wavelength 270 nm. Four major compounds were collected and identified through GC-MS analysis at 70 ev and with Rtx-5 MS column (60 m × 250 µm i.d. × 0.25 µm film thickness) using helium as a carrier gas. A thorough

literature survey was carried out to search for various bioactivities of the identified compounds (Iftikhar *et al.*, 2019).

RESULTS AND DISCUSSION

S. cumini fruit confer vital compounds that have been used traditionally to cure various diseases (Jagetia and Baliga, 2002). Previously, many pharmacological studies were carried out to explore their therapeutic potential against the pathogenic microbes (Migliato, 2005; Braga *et al.*, 2007). Entire plant parts warrant the most significant phytoconstituents like oxalic acid, geranyl acetone, maleic acid, tannins, ellagic acid, vernolic acid, stearic acid, guaicol, gallic acid, cineole, oleanolic acid, pinocarvone, isoquercetin, crategolic acid, cynidin glycoside, mycaminose, isorhamnetin 3-O-rutinoside, essential oils, myricetin, flavonoids, quercetin, gallotannin, betulinic acid, ellagic acid, ellagitannin, kaempferol, syzygiresinol, β -sitosterol, β -sitosterol-D-glucoside, friedelan3-a-ol and friedelin with antibacterial, antimicrobial, antifungal, antineoplastic, antipyretic, antidiarrheal, hypoglycemic, hypolipidemic, antioxidant, anti-inflammatory, anti-aging, chemo preventive, antitumor, antidepressant and gastroprotective properties (Ahn *et al.*, 2005; Meragelman *et al.*, 2005; Lima *et al.*, 2007). Recent studies have shown that fruit pulp is enriched with nutritive contents and minerals like calcium, potassium, phosphorous, sodium, zinc, iron, thiamine, ascorbic acid, niacin, mannose, fructose, maltose, galactose, alanine, glutamine, cysteine, tyrosine, asparagine, cinnamyl acetate, cinnamaldehyde, chrysanthem, nerol, linalool oxide, phenylethanol and phenylpropanal (Benherlal and Arumughan, 2007).

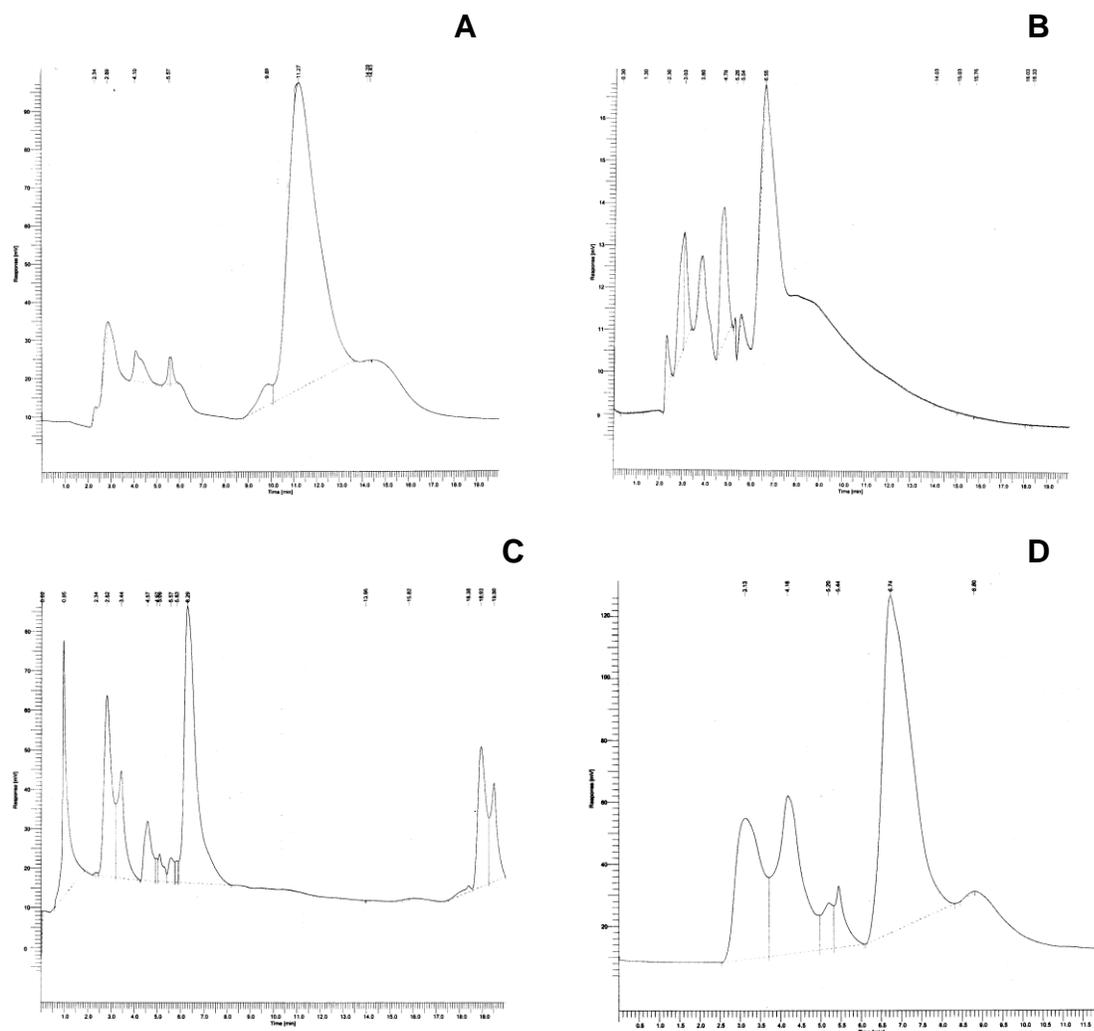


Fig. 1. HPLC chromatograms of sub-fractions of chloroform fraction of methanolic fruit extract of *Syzygium cumini* isolated by using PTLC.

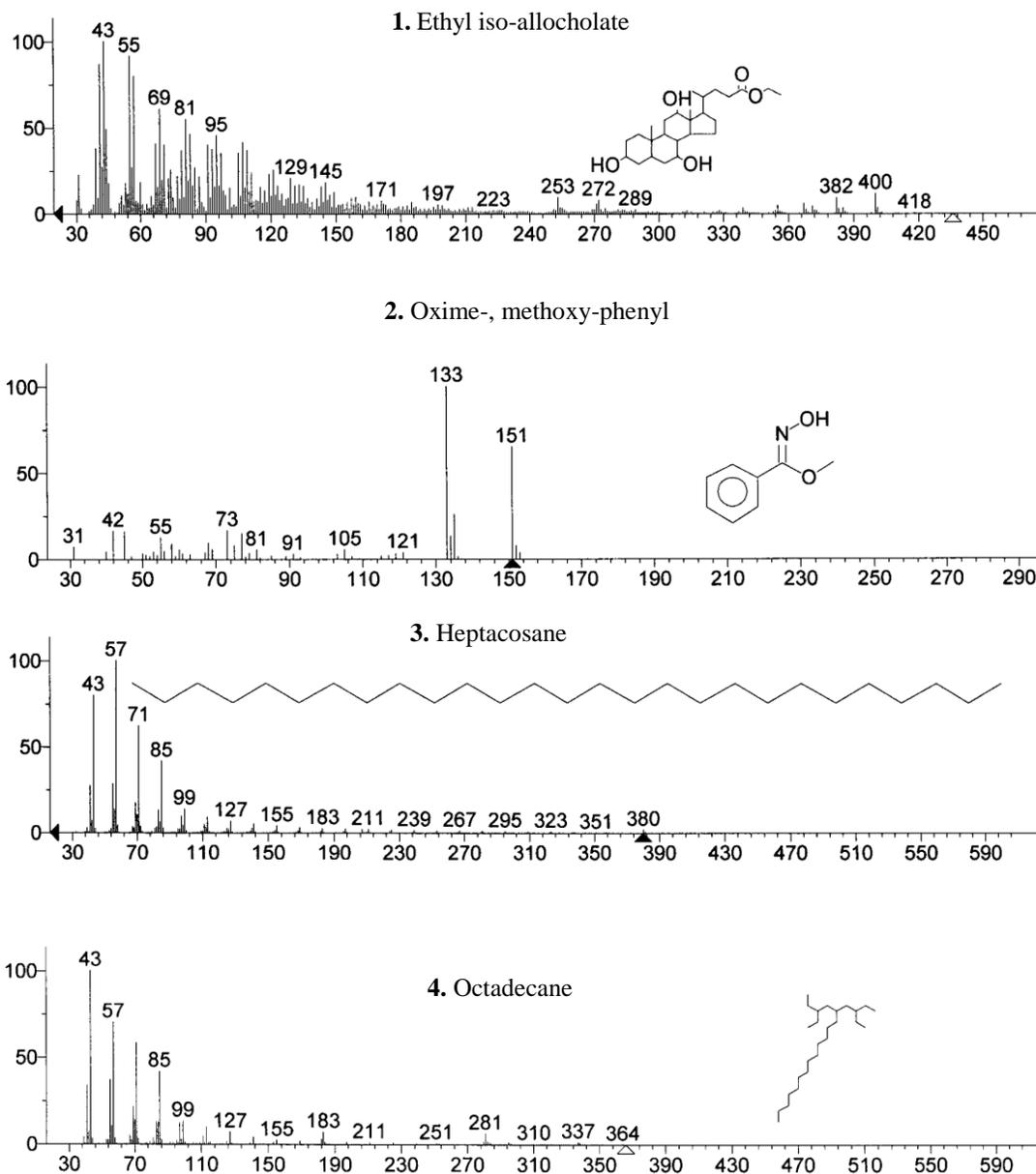


Fig. 2. Structures of compounds 1–4 isolated from chloroform fraction of methanolic fruit extract of *S. cumini*.

Table 1. Bioactivity of components of chloroform fraction of methanolic fruit extract of *S. cumini*.

| Comp. No. | Names of compounds | Bioactivity | Reference |
|-----------|------------------------|---|---|
| 1 | Ethyl iso-allocholate | Anti-inflammatory, antimicrobial, antiasthma, anti-arthritic, anticancer and diuretic | Hugar and Londonkar (2017); Sosa <i>et al.</i> (2016); Muthulakshmi <i>et al.</i> (2012); Daffodil <i>et al.</i> (2012) |
| 2 | Oxime-, methoxy-phenyl | Antioxidant, antifungal, antibacterial and antimicrobial | Altaee <i>et al.</i> (2017); Al-Jassaci <i>et al.</i> (2016); Altameme <i>et al.</i> (2015) |
| 3 | Heptacosane | Antioxidant and antifungal | Shobier <i>et al.</i> (2016); Vaithyanathan and Mirunalini (2015) |
| 4 | Octadecane | Antimicrobial | Arora <i>et al.</i> (2017); Ozdemir <i>et al.</i> (2004) |

Four compounds were identified in the present study. The first compound was ethyl iso-allochololate (**1**) with formula $C_{26}H_{44}O_5$ and molecular weight 436 (Fig. 2A). It was previously isolated from *Euphorbia lathyris*, a Chinese medicinal plant used traditionally as a substitute to synthetic chemical constituents. In nature, it is a steroid and possesses strong anti-inflammatory, anti-arthritic, antiasthma, antimicrobial, anticancer and diuretic activities (Sosa *et al.*, 2016). The second isolated compound was oxime-, methoxy-phenyl (**2**) with molecular weight 151 and molecular formula $C_8H_9NO_2$ (Fig. 2B). It was previously identified through GC-MS analysis of methanolic leaf extract of *Urtica dioica*. This compound exhibited antimicrobial and antioxidant properties (Altameme *et al.*, 2015). Moreover, it was tested against the fungal pathogenic strains namely *Fusarium* sp., *Mucor* sp., *Candida albicans*, *Penicillium expansum*, *Aspergillus flavus*, *A. fumigatus*, *A. niger* and *A. terreus*. It was found to be highly effective in reducing the growth of *A. fumigatus*, *A. terreus*, *A. flavus* and *Fusarium* sp. (Altaee *et al.*, 2017). Al-Jassaci *et al.* (2016) also worked on compound **2** and proved that it is an antibacterial against several clinical pathogenic bacterial strains *viz.* *Streptococcus pyogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae* and *Proteus mirabilis* (Table 1).

The third identified compound was heptacosane (**3**) with formula $C_{27}H_{56}$ and molecular weight 380 (Fig. 2C). It was also isolated by Vaithyanathan and Mirunalini, (2015) from the methanolic extracts of *Pergularia daemia* with antioxidant properties. Moreover, it was tested against *A. flavipes*, *F. oxysporum*, *F. solani* and *C. albicans* with promising antifungal activity (Shobier *et al.*, 2016). The fourth isolated compound was octadecane (**4**) with formula $C_{26}H_{54}$ and molecular weight 366 (Fig. 2D). It is a known hydrocarbon and was previously isolated from a nutritious grass *Cenchrus setigerus* hexane extract. It was reported very effective in inhibiting the growth of some plant pathogens (Ozdemir *et al.*, 2004; Arora *et al.*, 2017). The present study enlightens that the four compounds isolated from chloroform fruit extract of *S. cumini* through TLC followed by HPLC possess antioxidant, anti-inflammatory, antimicrobial, anticancer, antifungal, antibacterial and antiasthma properties.

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