Identification and Pharmacological Evaluation of *Syzygium cumini* Derived Fixed Oils

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Summary: *Syzygium cumini* L is an evergreen medicinal plant belonging to the family Myrtaceae, and is one of the important species of the genus *Syzygium*. *S. cumini* is traditionally used to treat various infections such as sore throat, bronchitis, dysentery, and other painful conditions. The aim of the current study was to evaluate fixed oil extracted from *S. cumini* for various *in vitro* and *in vivo* biological activities and identification of its fatty acids constituents. GC-MS analysis of the fixed oil revealed that the major constituents are palmitic acid methyl ester (56.45%) and stearic acid methyl ester (29.54%). Fixed oil isolated from *S. cumini* exhibited significant effect against *Staphylococcus aureus*, *Shigella flexenari*, and *Bacillus subtilis* with zone of inhibition of 28.09, 27.34, and 22.76 mm, respectively. In addition, the oil exhibited antifungal effect against *Aspergillus flavus*, *Candida glabrata*, *Microsporum canis*, and *Fusarium solani* with zone of inhibition of 80.77, 75.08, 70.98, and 65.56 mm, respectively. The oil also exerted analgesic potential at the dose of 10, 20, 40, and 60 mg/Kg with 35.44, 50.45, 65.98, and 80.34 % activity in acetic acid-induced writhing model. A mild muscle relaxant effect was noted in animal models. Taken all together, the fixed oil from *S. cumini* exhibits significant antimicrobial, antifungal and analgesic effects, which justifies the use of this plant in folk medicine.

Key words: *Syzygium cumini;* Fixed oil; GC-MS; Fixed oil; Antibacterial; Analgesic effects.

Introduction

The genus *Syzygium* belonging to family Myrtaceae is native to Australia and tropical America. The genus comprises 1100 species, distributed throughout native range extending from Asia, pacific to Africa. Its highest levels of diversity occur from Malaysia to northeastern Australia, where many species are still taxonomically unexplored or poorly known and much of its biodiversity remains undiscovered. Member plants of this family are considered rich sources of volatile oils, which added to it medicinal value [1]. In this respect, numerous fruits of the Myrtaceae family possess a rich history of use both as edibles and as traditional medicines in different ethnobotanical practices [2, 3]. *Syzygium cumini* L is an evergreen medicinal plant, which thrives in South Asian regions, including Bangladesh, Burma, India, Nepal, Pakistan, Sri Lanka, and Indonesia. It has also been cultivated and naturalized in Malaysia. The plant is considered sacred by Buddhists and Hindus, and commonly cultivated in close proximities of Hindu temples. It is considered sacred to Lord Krishna in Hindu religion [4].

S. cumini is a widely used medicinal plant that is employed in the treatment of various diseases including diabetes. Different parts of *S. cumini* especially fruits, seeds and stem bark possess promising pharmacological properties that can be used for the treatment of numerous diseases. Along this line, the bark of this plant is used in traditional medicines for the treatment of various infections such sore throat, bronchitis, dysentery, and other painful conditions. In addition, the plant, which is called Jambolan, is a source of important phytochemicals including anthocyanins, ellagic acid, glucoside, isoquercetin, myrecetin and kaemferol. Similarly, the plant seeds contain various alkaloids such as glycoside jambolin or antimellin, and jambosine. In this context, the seeds are known to halt the conversion of starch into sugar, and used to treat hypotension [5]. Furthermore, *S. cumini* stem contains various constituents such eugenin,

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β-sitosterol, butulinic acid, friedelin, gallic acid, kaemferol, myricetin, quercetin, epi-friedelanol as well as fatty ester of epi-friedelanol [6-10]. The fruit is astringent to the bowels and removes bad smell form mouth, biliousness, stomachic, astringent and used for treating chronic diarrhea, enteric disorders [11], diuretic and antidiabetic [12]. The bark is acrid, sweet, digestive, astringent to the bowels, anthelmintic and used for the treatment of sore throat, bronchitis, asthma, thirst, biliousness, dysentery and ulcers [13]. The seed extract is applied by local Indians to treat cold, cough, fever and skin problems such as rashes and the mouth, throat, intestines and genitourinary tract ulcers (infected by Candida albicans) [14]. Various parts of the plant are reported for antioxidant, anti-inflammatory, neuropsycho-pharmacological, antimicrobial, anti-bacterial, anti-HIV, antileishmanial and antifungal, nitric oxide scavenging, free radical scavenging, anti-diarrheal, antifertility, anorexigenic, gastroprotective and anti-ulcerogenic and radioprotective activities [15].

Based on literature cited in above mentioned discussion, the medicinal importance of *S. cumini* is evident in traditional medicine. However, there is limited experimental data regarding the effect of individual constituents of the plant. Therefore in the current study is focused on identification of fatty acids composition of fixed oils isolated from *S. cumini* and evaluating its antimicrobial, antifungal, analgesic and muscle relaxant effect.

Experimental

Chemicals

We obtained chemicals, reagents, and standards used throughout this work from commercial sources and used them as received. Solvents employed in the extraction process were of analytical graded. Diclofenac sodium, Diazepam, Distilled water, Streptomycin, paraquat, miconazole, permethrin, Muller-Hinton medium

Animals

BALB/c mice irrespective male female (22–25 g) both were used throughout this investigation. These animals were housed at constant room temperature (23–25ºC), given free access to standard diet and water, and were allowed to move freely in their respective cages. The standard conditions were applied for animal housing as mentioned in the "Animals By-Laws" with prior approval of the protocols by the ethical committee (EC/Ph/AWKUM-02156) of Department of Pharmacy Abdul Wali Khan University Mardan, KP, Pakistan. under standard laboratory conditions mentioned in the animal by laws approved by the ethical committee (SOU/Pharm-22) of Pharmacy Department, University of Swabi, Swabi, Pakistan.

Plant collection

Syzygium cumini barks were collected from Anbar, Swabi, KPK, Pakistan in July 2018. The taxonomist, Dr. Muhammad, Ilyas thoroughly identified-and authenticated, the plant specimens. The plant was deposited at the herbarium in Department. of Botany, University of Swabi., KP, Pakistan under voucher specimen number of "UOS/Bpt33".

Extraction of fatty acids and Preparation of Fames

Approximately 16 kg of bark of *S. cumini* was washed with water, shade dried, and powdered with the aid of heavy-duty grinding machine. The powdered plant material was allowed to be moistened in methanol for few days, filtered, and the solvent was removed at low temperature by means of a rotary evaporator to acquire a gummy precipitate (1.5 kg). The obtained extract was suspended in water, and subjected to column chromatography for elution with solvents of diverse polarities, to yield polar and non-polar fractions. The *n*-hexane fraction obtained in previous process was further subjected to chromatographic analysis using silica gel; the column was eluted with *n*-hexane and ethyl acetate which afforded yellow color oil. The extracted oil was filtered, to remove insoluble impurities, and then subjected to GC-MS analysis.

Since fatty acids extracted from the plant are not volatile, they were derivatized for GC-MS analysis according to a published procedure [16]. The reported method for converting the non-volatile fixed oil isolated from *S. cumini* into volatile fatty acids methyl ester was performed in the presence of BF_3 and methanol [17]. In this method, 0.1 mL of internal standard and 1.5 mL of 0.5 N NaOH solutions in methanol was combining with a known amount 25 mg of a fatty acid in a tube. The tube was sealed properly and heated for 5 min in a water bath. Then reaction mixture was allowed to cool and BF_3 solution (2.5mL) prepared in 10% methanol was added. The reaction mixture was then mixed with a saturated NaCl (5 mL) and two-times re-extracted with *n*-hexane. Then the *n*-hexane was removed via filtering through 0.45mic membrane filter and the resultant sample was injected into auto injector system of GC-MS.

Preparation of standard

The internal and external standard were prepared as stock solution that could further be diluted appropriately. Tridecanoic acid methyl ester (13.7g) was dissolved in *n*-hexane for further use as internal standard. On the other hand, each of standard 37 fatty acid ester

components (10mg) were dissolved in dichloromethane (DCM) (10ml) for further use as external standard.

Chromatographic separation of fatty acid methyl esters (FAMEs)

This analysis was carried out by means of gas [chromatography-mass spectrometry (GC/MS) using a spectrometer QP 2010. Chromatographic fractionations were accomplished by using a polyethylene glycol treated capillary column, with the dimensions (i.d; 0.35 mm, length; 30 m, thickness; 0.250 µm), and He as carrier gas. Then 1 µL of the sample along with the standard was injected into the GC column. Similar conditions were used as per a published procedure [17]. The mass spectra and retention time was compared to those of standards mas spectra in NIST library for identification of fatty acids components.

In vitro bioassay screening

Anti-bacterial activity

We used the agar well diffusion method to evaluate the antibacterial potential of the fixed oil extracted from *S. cumini*. In this assay, was used the Muller-Hinton medium (MHA). The prepared medium was incubated at 37 ^{0}C for 24-72 h. The sterile Petri Dish was dried and then 0.5 mL of broth culture, 0.5 mL of tested organism, and 15 mL of molten MHA were combined. The extracted oil sample and the standard drug (streptomycin) were applied. To ensure the correct diffusion of antibacterial agent in the medium, the incubation was performed for 1 h. The Petri Dish was incubated at 37 ^{0}C for a day, and the inhibition zone was analyzed and calculated in millimeter as per a reported method [18].

Insecticidal activity

The fixed oil extracted from *S. cumini* was assessed for *in vitro* insecticidal screening against *Tribolium castaneum*, *Rhyzopertha dominica*, and *Callosobruchus analis* by following a published procedure [19]. The stock solution of test sample was made by dissolving the oil extracted from *S. cumini* (2 mg) in 3 mL of methanol (3 mL). The sample µg/cm^2 was applied on the filter paper in a Petri plate, and was let to dry overnight (12 h). After 24 h, fifteen active and heathy insects of each species were combined in each plate with the methanol (control) and permethrin (the standard drug). Subsequently, the plates were incubated in the growing chamber for 24 h (conditions: 27 °C; relative humidity 50%).

Antifungal activity

To evaluate the antifungal effect of the fixed oil, a stock solution (2 mg/mL) of oil extracted from *S. cumini* was prepared by dissolving 5 mg oil in dimethyl sulfoxide (DMSO) (2 mL) and kept in freezer for further use. To determine the growth of fungal strains, sabouraud dextrose agar (SDA) media was used. SDA can be prepared by combining glucose, sabouraud and agar in distilled water in a slightly acidic medium (pH 5.5 to 5.6) and then sterilized by autoclaving [20]. Then the SDA medium was cooled down to 45–50 °C. Then the stock solution and SDA were combined to afford a finial concentration of 2 mg/mL. After seven days, the antifungal effect of tested oil sample and standard drug (miconazole) dissolved in DMSO was identified and measured as per standard procedures [20, 21]; the antifungal activity was recoded in terms of zone of inhibition in mm.

Phytotoxic activity

The phytotoxic activity of the oil against *Lamina minor* was assessed using fatty acids extracted from *S. cumini*. The stock solution was prepared by mixing 10 mg of oils in 1.5 mL of solvent. Furthermore, 5, 50, and 500 µL of the stock solution were transferred to several flasks to obtain solutions with concentrations of 10, 100 and 1000 µg/mL. All flasks were sterilized and then 20 mL of E. medium as well as standard drug (paraquat) which act as reference control were added to each flask. Afterwards, 10 plants with two to three fronds were placed in each flask and kept under standard conditions of circadian (12h light-dark cycle). On the seventh day, the numbers of fronds of plants in all flasks were observed and counted. The percent growth inhibitions was recoded according to a published procedure [19].

In vivo screening

Analgesics activity

In this assay, animals were randomly divided into several groups such as negative control (10 mL/kg, PO, DW), positive control (10 mg/kg, IP, Diclofenac), and tested groups (10, 20, 40, and 60 mg/kg). Animals in all groups were injected with 1% acetic acid solution (IP) after 30 min of the above treatments. After 10 min of the acetic acid injection, the writhings/contraction in abdominal muscle were counted during 10 min in each group (n = 8) of animals.

Muscle relaxation activity

Inclined plant test

To evaluate the effect of fixed oil for muscle co-ordination, a plane of two wood was used in such a way that an angle of 65^0 was obtained from the connection. Animals of the negative, positive, and treated groups

were administered with DW (10 mL/kg), diazepam (1 mg/kg), and fixed oil at tested doses of 10, 20, 40, and 60 mg/kg. After half minute, one minute and one & a half minute of the above treatment, animals were tested for the muscle coordination effect. The animals were allowed to hang from upper part of the inclined plane for 30 seconds. This is a modified form of our published method [22].

Traction test

In this test, a rubber coated metal wire with both ends fixed to stands, which rigidly supported it about 60 cm above the floor. The test was carried out at 30, 60, and 90 min durations after fixed oil administration to animals. The animals were allowed to hang for 5 s from their hind legs. The decrease in the duration of hanging was reflected as muscle relaxant effect and vice versa [22].

Statistical analysis

The percent values were calculated and expressed as the mean ± standard error of the mean (SEM). Then data of analgesic activity and muscle relaxant activity of standard and test samples were compared by subjecting to post Dunnett's multiple assessment screening and one way analysis of variance (ANOVA) using GraghPad-prsim-5, where differences were considered significant at $p \leq 0.05$.

Results and Discussion

GC-MS analysis

The GC-MS chromatogram depicted in figure 1, represented fixed oil of *S. cumini*. The analysis indicated the occurrence of saturated and unsaturated fatty acids in the oil sample (Table 1). Among the identified constituents, palmitic acid methyls ester was found abundantly (56.45.%) followed by steric acid methyl ester (29.54.%), oleic acid (7.35%), 9,12-octadecadienoic acid (2.25%), and margaric acid (1.65%). In addition, there were other acids where the concentration was less than 1%.

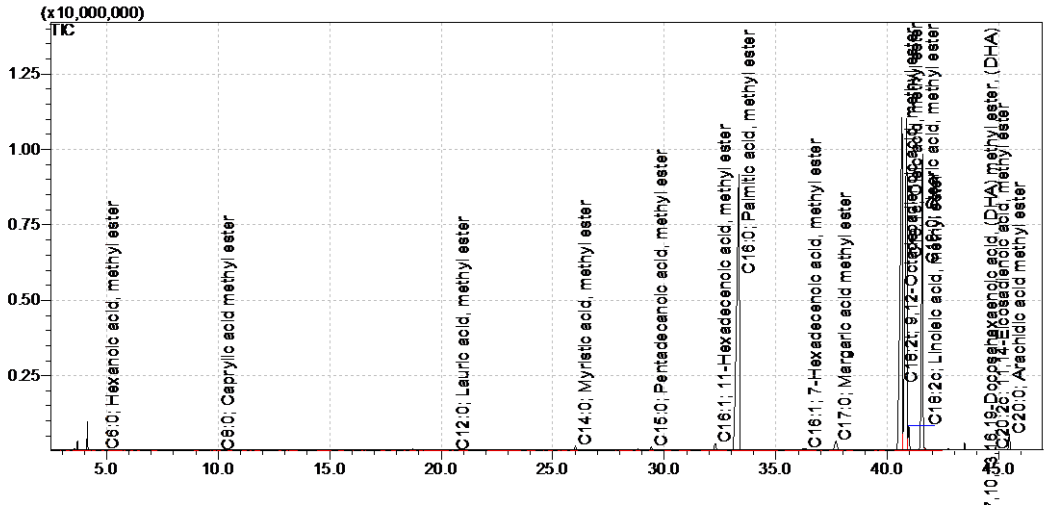

Fig. 1: GC-MS constituent quantification of-fixed-oil isolated from *S. cumini.*

Table-1: Quantities results of fixed oil extracts from *S. cumini* barks.

ID No.	Name of esteric of fatty acids	R. Time	Area	Conc.%
1	C6:0;hexanoic acid	4.825	7607	0.01
2	C8: Caprylic acid	10.011	1477	0.00
3	C10:0; Capric acid	15.482	1440	0.00
5	C12:0; Lauric acid	20.525	4275	0.01
8	C14:0; Myristic acid	26.013	243956	0.43
10	C15:0; Pentadecanooic acid	29.418	230589	0.41
11	C16:1; 7-Hexadecenoic acid	32.281	230589	0.20
12	C16:0; Palmitic acid	33.340	112457	56.45
13	C16:1; 7-hexadecenoic acid	36.325	320589	0.05
14	C17:0; Margaric acid	37.687	25516	1.65
16	C18:2t; 9,12-Octadecadienoic acid	40.533	934616	2.25
17	C18:1c; Oleic acid	40.858	1275537	7.35
18	C18:2C; Linolecic acid	40.952	4169146	0.27
20	C18:2C; Steric acid	41.574	154048	29.54
22	C22:6n3; 4,7,10,13,16,19-Docosahexaenoic acid (DHA)	44.236	16760224	0.00
24	C20:2c:11,14-Eicosadienoic acid	44.721	17043	0.03
26	C20:0; Arachidic acid	45.463	769099	1.36

Antibacterial effect

The fixed oil demonstrated variable effect against different tested bacterial species as shown in table 2. The maximum effect was against *B. subtilis* followed by *S. aureus* and *S. flexenari* with zone of inhibition 28.09, 27.34 and 22.76 mm respectively. The antibacterial effect was found to be less than standard drug streptomycin. The fixed oil did not inhibited the growth of *Pseudomonas aeruginosa* unlike streptomycin.

Table-2: Antibacterial activity of fixed oil extracts from *S. cumini* barks.

Name of bacteria	Zone of inhibition (mm)	
	Fixed oil	Streptomycin
Staphylococcus aureus	**27.34 ± 2.06**	**35.76 ± 0.23**
Shigella flexenari	**22.76 ± 1.98**	**30.09 ± 0.11**
Escherichia coli	**10.87 ± 2.09**	**32.23 ± 0.22**
Pseudomonas aeruginosa	-	**28.45 ± 0.43**
Bacillus subtilis	**28.09 ± 2.76**	**30.05 ± 0.02**

Antifungal effect

Our findings showed that the fixed oil extracted from *S. cumini* exhibits antifungal effect against tested species as given in Table-3. The maximum inhibition was observed against *A. flavus* (80.77 mm) when compared with the standard amphotericin (26.43 mm) at minimum inhibitory concentration. Additionally, the fixed oils inhibited the growth of *C. glabrata*, *M. canis*, and *F. solani* with zone of inhibition of 75.08, 70.98, and 65.56 mm, respectively. The standards antifungal miconazole (0.2 mg/mL) inhibited the growth of the aforementioned strains with zone of inhibition of 112.11, 100.00, and 87.98 mm, respectively.

Table-3: Antifungal activity of fixed oil isolated from *S. cumini* barks.

Name of fungi	Zone of inhibition (mm)		
	Fixed oil	Control	Standard Drug (mm)
Candida glabrata	**75.08 ± 2.65**	**100.00**	**Miconazole = 112.11**
Microsporum canis	**70.98 ± 2.97**	**100.00**	**Miconazole = 100.00**
Aspergillus flavus	**80.77 ± 2.97**	**100.00**	**Amphotericin = 26.43**
Fusarium solani	**65.56 ± 3.01**	**100.00**	**Miconazole = 87.98**

Insecticidal and phytotoxic effects

Based on our observations, the fixed oil from *S. cumini* did not exhibit any significant insecticidal or phytotoxic effects as shown in Tables 4 and 5.

Analgesic effect

Shown in Table 6 are results of our study of the analgesic effect of fixed oil extracted from *S. cumini* barks. Our findings reveal that the fixed oil exhibits dose-dependent inhibition of writhing. Fixed oils were used in doses of 10, 20, 40, and 60 mg/kg, which produced percentage inhibition of 35.4, 50.5, 66.0, and 80.3%, whereas the standard diclofenac sodium (10 mg/kg) caused 84.5 % inhibition. These results indicate that the most significant effect was observed at 60 mg/kg dose followed by 40 mg/kg.

Muscle relaxant effect

Displayed in Table 7 are our findings of the muscle relaxant effect of *S. cumini*, using the muscle inclined plane and Traction test models. Results showed a uniform effect in both models. The maximum effect (38.87 %) was observed when the oil was given at the dose of 60 mg/kg after 90 min of administration. In addition, a mild muscle relaxant effect was observed at higher doses.

Table-4: Insecticidal activity of fixed oil from the barks of *S. cumini*.

Name-of-Insect	%Mortality		
	Positive control	Negative Control	Fixed oil
Rhyzopertha dominica	**100.00**	-	-
Tribolium castaneum	**100.00**	-	-
Clinocottus analis	**100.00**	-	-

Table-5: Phytoxicity of fixed oil from *S. cumini* barks.

Sample	Concentration- (µg/ml)	Fronds-survived	Fronds-died	% Growth-Regulation
Fixed oil	**1000**	**7.09 ± 0.23**	**13.87 ± 0.87**	**65.22**
	100	**8.00 ± 0.27**	**12.45 ± 0.65**	**60.54**
	10	**10.65 ± 0.11**	**10.00 ± 0.87**	**50.70**

Table-6: Analgesic activity of fixed oil extracts obtained from *S. cumini* barks.

Treatment	Dose (mg/kg)	Percent inhibition of writhing (10 minutes duration)
Saline	**10 mL/kg**	-
Diclofenac sodium	**10**	**84.51 ± 1.44*** **
Fixed oil	**10**	**35.44 ± 2.55**
	20	**50.45 ± 2.00** **
	40	**65.98 ± 1.88** **
	60	**80.34 ± 1.45*** **

Table-7: Muscle relaxation effect of fixed oil extracted from *S. cumini* barks.

Group	Dose (mg/kg)	Inclined plane test (% activity)			Traction test (% activity)		
		30-min	60-min	90-min	30-min	60-min	90-min
Distilled water	10 mL	0.00 ±0	0.00 ±0	0.00 ±0	0.00 ±0	0.00 ±0	0.00 ±0
Diazapam	1	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
Oil sample	10	19.09 ± 1.09	24.20 ± 1.66	26.87±1.06	19.32 ± 1.88	20.34 ± 1.34	19.54 ± 1.66
	20	23.23 ± 1.30	28.43 ±.54	29.34 ±.1.02	22.11±.87	23.54 ± 1.91	22.90 ± 1.20
	40	27.96 ± 1.11	31.32 ± 1.32	33.11 ±1.00	27.23±1.54	28.98 ±1.22	27.81 ± 2.76
	60	32.3 ± 1.32	36.99 ± 1.07	38.87 ± 1.00	32.03 ± 1.12	33.09 ± 1.09	32.08 ± 1.22

Results obtained are the-mean ± standard-error-of-the-mean- (SEM) for all animals, tolerance to thermal stimuli in sec. Data were subjected to ANOVA followed by Dunnets screening model.

Medicinal plants have been used in the healthcare systems of different cultures to treat diseases and disorders for many years; these plants could be used as complimentary or alternative medicine. The pharmacological effect of medicinal plants is due to the presence of agonistic and antagonistic chemical constitutes. Due to this unique chemical accumulation, the plants have the potential of multi-indications. The allopathic systems of medicines have numerous options with respect to the chemical diversity for the treatment or management of various disorders. However, due to adverse drug reactions of allopathic systems and side effects of current synthetic drugs, patients are reluctant to use modern medicines. In addition, despite the advent of technological and pharmaceutical improvements over the few decades, we are still unable to eradicate numerous disorders such as hypertension and diabetic mellitus among others. Accordingly, there is a need for a safe, effective, and economical therapeutic agents for such conditions and natural products are the best option to be explored.

The major constituents of fixed oil extracted from the bark of *S. cumini* were found to be palmitic acid (56.45%), steric acid (29.54%), oleic acid (7.35%), 9,12-octadecadienoic acid (2.25%), and margaric acid (1.65%). Our findings from this study reveal that the fixed oil of *S. cumini* demonstrated significant effects against some gram positive bacterial strains, including *Staphylococcus aureus*, *Bacillus subtilis* and gram negative bacteria including *Shigella flexenari*. The *S. aureus* and *B. subtilis* is part of our GIT normal flora and sometimes might be pathogenic, and can cause several infections [23-25]. The resistance against *Staphylococcus aureus*, especially methicillin resistance *Staphylococcus aureus* (MRSA), emerge the need for new safe and effective antibiotics. Based on our results, it will be worth trying to test this fixed oil against MRSA infections. Furthermore, our findings significantly justify the use of *S. cumini* in folk medicine to treat infections such as sore throat, bronchitis, dysentery, ulcers, and chronic diarrhea [references]. These infections are mostly due to gram-positive bacteria, and the fixed oil acts against these pathogens.

In addition, our results showed that the fixed oil is effective against fungi especially *Aspergillus flavus, C. glabrata*, *M. canis*, and *F. solani*. The seed extract of *S. cumini* traditionally applied in India for *Candida albican* caused infections of skin, mouth, throat and genitourinary tract [14]. The antifungal potential of fixed oils isolated from *S. cumini* is experimentally validated and expanded. The *S. cumini* derived fixed oils were screened for insecticidal and phytotoxic effects and did not exhibit profound effects.

Similarly, the significant results of fixed oil in acetic acid-induced writhing test are also worth mentioning. Acetic acid induces the release of inflammatory mediators such as prostaglandins and bradykinins, thereby stimulating the pain receptors and induces algesia [26]. These findings imply that the fixed oil obtained from *S. cumini* could be used for the treatment or management of various painful conditions, probably by inhibiting cyclooxygenase pathway or by producing antioxidant effect. Our data is in correlation of the findings of other researchers reported that *S. cumini* has multi uses in the traditional system of medicine such as analgesic, diabetic, blood purifier, digestive, and antimicrobial [10]. The antioxidants have potential to subside inflammatory response, the anthocyanin, one of the major chemical constituent of *S. cumini* [27], which is one of the best antioxidants [10]. Therefore, this compound could be screened against all traditional uses of this plant. Finally, a topical formulation of the fixed oil isolated from *S. cumini* can be tested for topical analgesia and antimicrobial actions. The fixed oil also demonstrated mild muscle relaxant effect. Taken all together, the fixed oil exerted antimicrobial, antifungal, analgesic and muscle relaxant effects.

Conclusions

In conclusion, our data suggest that bark extracts from *S. cumini* has a diverse fatty acids composition, and display remarkable biological activities such as antibacterial, antifungal, analgesic and muscle relaxation effect. In this respect, fatty acids are key components of the human body having biological and structural functional rule. Our findings showed the

presence of considerable amounts of palmitic acid (56.45%) and stearic acid (29.54%) in the *S. cumini* bark extract; these acids could be used in the pharmaceutical and food industry. The folk medicinal uses of *S. cumini* may be explained by findings in our study. However, more thorough studies need to be conducted to ensure the efficacy and safety of the active components of this plant. These fixed oils could further be formulated for topical application in conditions such as skin disorders caused by fungi and muscular pain.

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