Evaluating the antimicrobial activity, antioxidant potential and GC-MS based chemical characterization of *Cannabis sativa* found in Pakistan

ANAM AMIN SHAMI1, TASBEEHA MARJAN1, SYED ALI RAZA2, HAMID MUKHTAR1, MUHAMMAD WASEEM MUMTAZ3, NEELAM CHAUDHARY4*, MUHAMMAD TAYYAB AKHTAR1*

1 Institute of Industrial Biotechnology
GC University
Lahore, Pakistan

2 Laboratories of Water Resources Zone
Irrigation Department
Lahore, Pakistan

3 Department of Chemistry
University of Gujrat
Gujrat, Pakistan

4 Department of Continuing Education
University of Agriculture Faisalabad
Faisalabad, Pakistan

*corresponding authors: e-mail: tayyabakhtar@hotmail.com; neelam.imperial@gmail.com

Summary

Introduction: *Cannabis* is a dioecious plant belonging to the family Cannabaceae.

Objective: The present work was conducted to study the antimicrobial activity, antioxidant potential and GC-MS-based metabolite profiling of *Cannabis*.

Methods: The antimicrobial activity of *Cannabis* extract was assessed through disc-diffusion method. The anti-oxidant potential of CEO was checked through DPPH assay and FRAP assay. We also assessed the total phenolic (TPC) and flavonoid content (TFC) of the extract. The phytocompounds present in the *Cannabis* extract were characterized by using GC-MS.
Results: The antimicrobial assay revealed significant activity against *Pseudomonas*, *Staphylococcus aureus* and *Klebsiella pneumoniae*, whereas moderate antioxidant activity was shown by *Cannabis* extract, having 76.54% inhibition in DPPH assay and 56.10% inhibition in FRAP assay. TPC and TFC were 26.77 mg GAE/g D.E. and 34.45 mg QE/g D.W, respectively. The chemical characterization of *Cannabis* extract by GC-MS shows the presence of cannabinoids, terpenes, aliphatic compounds, alkanes and sesquiterpenes.

Conclusion: The current study revealed that *Cannabis* found in Pakistan, possesses medicinally potent metabolites responsible for its anti-microbial and antioxidant potential.

Key words: *Cannabis sativa*, metabolite profiling, antioxidant potential, antimicrobial activity, TPC and TFC, phytochemicals, GC-MS

Słowa kluczowe: *Cannabis sativa*, profilowanie metaboliczne, potencjał antyoksydacyjny, aktywność przeciwbakteryjna, TPC i TFC, związki czynne, GC-MS

INTRODUCTION

Medicinal plants have been used in folk medicine for thousands of years. Nowadays these plants have gained recognition, owing to the presence of biologically active secondary metabolites [1]. Among secondary metabolites, volatile mixtures of compounds (also known as essential oils (EOs)) have gained attention, because of their biological activities. Many reports have described the applications of these plant-based products (EOs) as anti-microbial, anti-oxidant and anti-inflammatory agents [2].

*Cannabis*, a medicinal plant, belongs to the family *Cannabaceae*. The most important species of *Cannabis* is *Cannabis sativa* (*C. sativa*). Generally, this plant has three major cultivars i.e. *Cannabis sativa*, *Cannabis indica*, and *Cannabis ruderalis*, native to Central Asia [3]. For centuries, *Cannabis* has been used both for recreational and therapeutic purposes [4]. *Cannabis* products have different names in various regions of the world: Bango in Sudan, Marijuana in America, Bhang in India and Pakistan, Kif in North Africa, Hashish in Middle East, Grifa in Mexico and Macohna in some parts of South America [5]. In Pakistan, *Cannabis* grows naturally as a wild plant in rural and northern areas. It is cultivated on a low scale in the country as a crop due to its use as a recreational drug [6].

Besides hallucinogenic properties, *Cannabis* is a reservoir of bioactive phytochemicals. These biologically active phytochemicals have many medicinal and non-medicinal applications. The diverse phytochemistry of *Cannabis* has brought the attention of pharmacologists to retrieve its pharmacological power [7].

Over 500 compounds, including cannabinoids, terpenoids and flavonoids have been extracted and identified from *C. sativa* [8]. Cannabinoids are the most studied phytochemicals among all reported in *C. sativa*. According to recent studies, over 120 different cannabinoids are known [9]. These phytocannabinoids are further subdivided into 11 subclasses, inclusively cannabigerol (CBG-type), (–)-Δ⁸-tetrahydrocannabinol (Δ⁸-THC-type), cannabidiol (CBD-type), cannabichromene (CBC-type), cannabinol (CBN-type), (–)-Δ⁴-tetrahydrocannabinol (Δ⁴-THC-type), cannabicyclol (CBL-type), cannabinodiol (CBND-type), cannabinol (CBND-type), cannabitriol (CBT-type) and miscellaneous type [9-10]. The two preeminent terpenophenolic compounds found in *C. sativa* are THC and CBD. The former has a psychotic effect, while the latter does not possess psychotic activities [11]. These phytocannabinoids possess potent therapeutic properties, and are widely used as analgesic, antiemetic, antioxidant and neuroprotective agents [12]. *Cannabis* essential oil (CEO) extracted from different parts of the plant have been reported to have various pharmacologically important compounds viz. myrcene, terpinolene, limonene, α-pinene, β-caryophyllene and β-pinene [13].

There is little information available regarding the antimicrobial and antioxidant potential of *Cannabis* species present in Pakistan. The purpose of the study was to obtain bioactive essential oil from wild *C. sativa* found in Pakistan. The major focus of the study was the chemical characterization of *Cannabis* essential oil, assessing its antimicrobial and antioxidant potential.

MATERIAL AND METHODS

Sample collection

The wild *Cannabis sativa* (trichomes) were harvested from Khanaspur (Murree, Pakistan). The plant was identified by Dr. Zafar Siddique, the botanist at
Decarboxylation of plant material

The decarboxylation of the plant material (Cannabis Trichomes) was performed by preheating the plant at 120°C in a hot air oven for 7 hours. Decarboxylation was primarily performed to transform acidic cannabinoids (i.e. tetrahydrocannabinolic acid (THCA) or cannabidiolic acid (CBDA) into more stable substances i.e. tetrahydrocannabinol (THC) and cannabidiol (CBD) [14]. The plant material was then stored in a well-ventilated place for further use.

Extraction of oil

Dried and decarboxylated female flower tops (10 g) were extracted thrice by transferring to an Erlenmeyer flask containing 125 ml of n-hexane. Each extraction was performed by shaking the plant sample for 15 mins and later sononating it for 5 mins in order to increase the cannabinoids extraction rate. Finally, all three extracts were pooled and then filtered using Whatman filter paper No. 1. The filtrate acquired was concentrated using a rotary evaporator at 40°C. The concentrated extract was then dissolved in 5 ml of methanol and stored at −20°C. A working solution of 25 mg/ml was prepared from the stock and was used for antimicrobial testing.

Antimicrobial activity

Antimicrobial activity of the tested strains was assessed by using the disc diffusion method [15]. Bacterial strains were cultured on nutrient agar plates at 37°C while fungal strains were cultured on potato dextrose agar plates at 30°C. A single bacterial and fungal colony was inoculated in nutrient broth and potato dextrose broth, respectively. A sterile plate was spread with 100 μl of broth and dried. Sterile discs were loaded with 6 μl (25 mg/ml) of plant extract and allowed to stand for 20 minutes for maximum absorption. A disc was then placed on lawn cultured plates under aseptic conditions. The plates were then incubated for 24 hrs for bacterial culture and 72 hrs for fungal culture. Sensitivity was checked by measuring the diameter of the inhibition zone.

Antioxidant activity

DPPH radical scavenging assay

Antioxidant activity was estimated using DPPH scavenging assay [16] with some modifications. Different concentrations of Cannabis essential oil were prepared by dissolving CEO in methanol and varying the concentration from 100 to 500 μg/ml. An aliquot of 5 μl of CEO was then dissolved in methanol solution of DPPH (0.004 w/v%). The solution was incubated in a dark place at room temperature for at least 30 mins. After incubation, the absorbance of samples was recorded at 517 nm. Butylated Hydroxytoluene (BHT) was taken as a positive control. All the experimental measurements were performed in duplicates. The inhibition percentage was found using the following formula:

\[ \text{% inhibition} = \left( \frac{A_{\text{Sample}} - A_{\text{Control}}}{A_{\text{Sample}}} \right) \times 100 \]

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed to measure the total antioxidant capacity of the CEO. An assay was performed by adding the sample (100 μl) to 3.4 μl of the FRAP reagent in an Eppendorf tube and the contents were mixed thoroughly. The solution was incubated at room temperature in a dark place for half an hour. The absorbance was then recorded at 593 nm and the inhibition percentage was calculated [17].

\[ \text{FRAP % inhibition} = \left( \frac{A_{\text{Sample}} - A_{\text{Control}}}{A_{\text{Sample}}} \right) \times 100 \]
Determination of total phenolic contents (TPC)

Total phenolic content of essential oil was estimated using Folin-Ciocalteu reagent [18]. The reaction mixture was prepared by adding 1 ml of Folin-Ciocalteu reagent, 0.1 ml of oil extract and 2 ml of Na₂CO₃ (10%), then raised the volume up to 5 ml using double distilled water. Later, the sample was incubated for 2 hours at room temperature and optical density was measured at 765 nm by using a spectrophotometer. The readings were compared with the gallic acid standard curve and expressed as milligram gallic acid equivalent per gram of dried extract (mg GAE/g D.E.).

Determination of total flavonoid contents (TFC)

The total flavonoid content of the CEO was determined by protocol, as mentioned in [19]. Methanolic CEO extract (100 μl) was diluted by adding 4 ml of distilled water. In the diluted mixture of CEO, 0.6 ml of NaNO₂ (5%) and 0.6 ml of AlCl₃ (10%) was added and the reaction mixture was incubated for 10 min. at a room temperature. After incubation, 1M NaOH (4 ml) was added to the reaction mixture. Finally, the resultant mixture's volume was raised up to 20 ml and then allowed to stand for 20 minutes. Afterwards, the optical density was measured at 510 nm. The results were expressed as mg quercetin equivalent per gram of dry extract (mg QE/g D.E.).

GC-MS analysis

The phytochemical analysis was performed using Gas Chromatography-Mass Spectrometry (GC-MS). The methanolic CEO was filtered using a syringe filter and then subjected to GC-MS analysis. The GC-MS analysis was performed by using GC-MS QP Shimadzu Japan 2010, column db 5 having 0.15 mm column diameter. Helium was used as a carrier gas whose flow rate was kept at 2 ml/min during the process. The spectrum was then obtained and interpreted.

Ethical approval: The research conducted is not related to either human or animal use.

RESULTS AND DISCUSSION

The antimicrobial activity of Cannabis essential oil (CEO) was tested against six bacterial and four fungal strains, using the disc diffusion method. The observed results for bacteria and fungi are shown in fig. 1, 2. The methanolic extract from the trichome's of C. sativa exerted notable results against S. aureus (18 mm). The CEO shows moderate activity against P. aeruginosa (17 mm), Klebsiella pneumonia (16 mm), and B. subtilis (14 mm). While, E. coli and Methicillin-resistant S. aureus (MRSA) were found to be insensitive against CEO. All the fungal strains

Figure 1.

Inhibitory zones produced by CEO against A) Pseudomonas; B) S. aureus; C) Klebsiella; D) MRSA; E) B. subtilis; F) A. fumigatus
were also found insensitive against the CEO (fig. 3). The DPPH method was employed for estimating the antioxidant properties of CEO at different concentrations (Fig. 4). The findings revealed that CEO exhibited moderate antioxidant ability in comparison to BHT (which is a highly antioxidant compound). The highest percentage of DPPH reduction (76.54%) was observed at 500 μg/ml. The data shows that, by increasing the concentration of CEO, the antioxidant activity increases gradually.

The FRAP assay was performed to measure the antioxidant power of the CEO (fig. 5). This method employs the reducing ability of Fe\(^{3+}\) to Fe\(^{2+}\) with color change due to complex formation. The results unveiled that FRAP assay follows the same trend as DPPH assay, showing maximum reducing ion power (56.01%) at 500 μg/ml. Polyphenolic compounds were considered to be the key components in determining the antioxidant potential of a substance. In the present study, the TPC in CEO was 29.77 mg GAE/g D.E, while TFC was 34.45 mg QE/D.W.

The chemical fingerprinting of the CEO was performed by GC-MS. The identified compounds and fragmentation is shown in table 1, GC-MS
Evaluating the anti-microbial activity, anti-oxidant potential and GC-MS based chemical characterization of Cannabis sativa found... 39

spectra, fragmentation pattern and chemical structures are shown in supplementary figure 1. Along with terpenes, diterpenes, sesquiterpenes and aromatic compounds, cannabinoids were identified as major compounds present in CEO.

Our study demonstrated that Cannabis essential oil was effective in hampering the growth of opportunistic and etiologic agents. The antimicrobial effect of CEO was due to the presence of terpenes, sesquiterpenes, cannabidiol and dronabinol [20]. The research conducted by Okwu and Uchenna in 2011 suggested that dronabinol, the bioactive component present in the extracts of Cassia alata was responsible for the antimicrobial effect against Klebsiella, S. aureus, E. coli, P. aeruginosa, C. albicans and A. niger [21].

Ali et al. [5] studied antimicrobial potential of methanolic extract of Cannabis (seeds) and found pronounced bactericidal activity against Bacillus subtilis (29 mm), gram-negative E. coli (15 mm), and Pseudomonas (16 mm), while lower activity was found against Staphylococcus aureus (12 mm). Compared to their results, this study has found CEO to be moderately active against Pseudomonas aeruginosa (17 mm) and Bacillus subtilis (14 mm). The difference in antimicrobial activity might be
### Table 1.

List of identified compounds in *Cannabis* essential oil by GC-MS

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Name of compound</th>
<th>Molecular weight</th>
<th>Fragmentation pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,3-Dimethylpentane</td>
<td>100</td>
<td>85, 71, 43, 41, 27</td>
</tr>
<tr>
<td>2</td>
<td>Hexanol</td>
<td>102</td>
<td>70, 43, 27</td>
</tr>
<tr>
<td>3</td>
<td>Pentafluoropropionic acid</td>
<td>262</td>
<td>147, 100, 70, 41, 30</td>
</tr>
<tr>
<td>4</td>
<td>1-Hydroperoxyhexane</td>
<td>118</td>
<td>147, 97, 70, 41, 30</td>
</tr>
<tr>
<td>5</td>
<td>alpha.-Ribazole dibenzoate</td>
<td>486</td>
<td>409, 365, 244, 217, 175, 123, 105, 91, 77, 43</td>
</tr>
<tr>
<td>7</td>
<td>Diethyl phthalate</td>
<td>222</td>
<td>194, 177, 163, 149, 132, 105, 76, 50, 27</td>
</tr>
<tr>
<td>8</td>
<td>Phthalic acid</td>
<td>234</td>
<td>176, 149, 132, 122, 105, 93, 76, 50, 26</td>
</tr>
<tr>
<td>9</td>
<td>2-Propionylbenzoic acid</td>
<td>178</td>
<td>160, 149, 133, 104, 76, 50, 27</td>
</tr>
<tr>
<td>10</td>
<td>2-Acetylbenezic acid</td>
<td>164</td>
<td>164, 149, 121, 93, 65, 39</td>
</tr>
<tr>
<td>11</td>
<td>Phytol</td>
<td>296</td>
<td>196, 140, 123, 95, 71, 57, 41, 39</td>
</tr>
<tr>
<td>12</td>
<td>Cyclopentanecarboxylic acid</td>
<td>248</td>
<td>203, 161, 145, 115, 107, 91, 67, 41, 33</td>
</tr>
<tr>
<td>13</td>
<td>Neoisolongifolene, 8-bromo-</td>
<td>282</td>
<td>203, 187, 175, 147, 119, 91, 69, 41</td>
</tr>
<tr>
<td>14</td>
<td>Neoisolongifolene, 8-chloro-</td>
<td>238</td>
<td>223, 203, 175, 147, 119, 91, 69, 41</td>
</tr>
<tr>
<td>15</td>
<td>Cycloisolongifolene, 7-bromo-</td>
<td>282</td>
<td>255, 203, 187, 161, 147, 119, 91, 69, 41</td>
</tr>
<tr>
<td>16</td>
<td>Dihydrocoumarin</td>
<td>218</td>
<td>203, 189, 175, 159, 144, 115, 91, 65, 41</td>
</tr>
<tr>
<td>17</td>
<td>Dronabinol</td>
<td>314</td>
<td>271, 246, 231, 207, 174, 147, 121, 91, 67, 41</td>
</tr>
<tr>
<td>18</td>
<td>Resorcinol</td>
<td>314</td>
<td>295, 271, 246, 231, 207, 174, 147, 121, 91, 67, 43</td>
</tr>
<tr>
<td>19</td>
<td>Tetrahydrocannabinol</td>
<td>314</td>
<td>299, 285, 271, 243, 231, 201, 174, 147, 119, 91, 69, 41</td>
</tr>
<tr>
<td>20</td>
<td>Cannabinol</td>
<td>332</td>
<td>299, 271, 243, 231, 217, 193, 150, 121, 91, 69, 43, 41</td>
</tr>
<tr>
<td>21</td>
<td>Stigmast</td>
<td>412</td>
<td>314, 299, 271, 245, 217, 149, 123, 95, 82, 69, 55, 41</td>
</tr>
<tr>
<td>22</td>
<td>Docosane, 2,21-dimethyl</td>
<td>338</td>
<td>323, 295, 239, 211, 183, 155, 127, 99, 85, 71, 43, 41</td>
</tr>
<tr>
<td>23</td>
<td>Tricosane, 2-methyl</td>
<td>338</td>
<td>323, 295, 239, 211, 183, 155, 127, 99, 85, 71, 43, 41</td>
</tr>
<tr>
<td>24</td>
<td>2-Methylpentadecane</td>
<td>226</td>
<td>211, 183, 155, 127, 90, 85, 71, 43, 41</td>
</tr>
<tr>
<td>25</td>
<td>Nonadecane, 2-methyl</td>
<td>282</td>
<td>267, 239, 183, 155, 127, 99, 85, 43, 41</td>
</tr>
<tr>
<td>26</td>
<td>Eicosane, 2-methyl</td>
<td>296</td>
<td>281, 253, 211, 183, 155, 127, 99, 85, 71, 43, 41</td>
</tr>
<tr>
<td>27</td>
<td>Hexadecane</td>
<td>226</td>
<td>183, 155, 127, 99, 85, 71, 43, 41</td>
</tr>
<tr>
<td>28</td>
<td>Tridecane, 2-methyl</td>
<td>198</td>
<td>183, 155, 127, 99, 85, 71, 43, 41</td>
</tr>
</tbody>
</table>
Evaluating the anti-microbial activity, anti-oxidant potential and GC-MS based chemical characterization of Cannabis sativa found...

due to the difference in the method of extraction and plant parts used to test antimicrobial activity. Moreover, Ali et al. [5] also reported high activity of CEO against Pseudomonas (23–28 mm inhibition zone), unlike in the present study in which the zone of inhibition was about 17 mm. The reasons may include the difference of Pseudomonas strain and different methods used to find antimicrobial activity as previously cup agar plate method was followed, contrary to the disc diffusion method used in the present study. Furthermore, the nature of extracts can also be a reason for the difference, as we used hexane extracts, while Ali et al. [5] extracted plant samples with petroleum ether and methanol [5]. Likewise, Borchardt et al. [22] found pronounced antimicrobial activity of CEO against C. albicans, S. aureus, E. coli, and P. aeruginosa using disc diffusion method. Our data also shows strong activity against S. aureus and Pseudomonas, however, activity against E. coli is different as smaller inhibition zones against E. coli (6 mm) were observed as compared to the zones reported by Borchardt et al. [22] which were greater than 20 mm. The difference in the antimicrobial activity of essential oil against the studied microorganisms is primarily attributed to the difference in the qualitative and quantitative constituents of the oil. Moreover, the chemical composition of the microbes’ cell structure also plays an important role in the activity of essential oils. The presence of an outer membrane in the Gram-negative bacteria hinders the activity of essential oils making them more resistant.

Essential oils were reported to have notable anti-oxidant properties [23]. The antioxidant potential of essential oils is mainly due to the presence of monoterpenes. Evidence related to the antioxidant property of CEO is in its infancy. We have found moderate antioxidant property of CEO. Nafis et al. [24] reported the antioxidant potential of CEO was mainly due to the presence of (E)-caryophyllene and caryophyllene. Whereas neither of the constituents were identified in our sample, so the antioxidant activity of our sample might be attributed primarily to the presence of cannabidiol and tetrahydrocannabinol. As previously, the antioxidant potential of Cannabis extracts was credited to the presence of tetrahydrocannabinol and cannabidiol [25]. Frassinetti et al. (2018) found antioxidant activity of CEO from seeds and sprouts by using DPPH assay [26]. The highest percent inhibition of DPPH recorded by the CEO was 40% and 52% from seeds and sprouts, respectively. While in the current study, extract from Cannabis trichomes shows 76.54% inhibition, which is higher than seeds and sprouts found by Frassinetti et al. [26]. Likewise, Smeriglio et al. [27] also revealed higher antioxidant capacity of commercially available Cannabis seed oil than the activity found in the current study [27]. The difference in the antioxidant potential is possibly due to the variation in the concentration of bioactive compounds, temperature, type of substrate, and the physical state of the system.

Polyphenols (phenolics and flavonoids) are also well known to exhibit antioxidant properties [28]. So, the assessment of total phenolic contents (TPC) and total flavonoid contents (TFC) was done to further confirm the antioxidant potential of the CEO. The total flavonoid content of the CEO under study was in compliance with the previously published data by Agarwal et al. [29]. They reported TFC content of Cannabis trichomes extract (28.173 mg) which is similar to our study. Furthermore, Kitrytė et al. [30] shows TPC from Cannabis extract fractions obtained by supercritical CO2 method were in the range of 1.3–23 mg GAE/g D.E. [30] which is also quite close to the TPC of the present study that is 26.77 mg GAE/g D.E. Frassinetti et al. studied the TPC and TFC content of Cannabis extract from seeds and sprouts that were 6 mg GAE/g D.E. and 5.32 mg QE/g D.W., respectively. While we have found 26.77 mg GAE/g D.E. and 34.45 mg QE/g D.W. of TPC and TFC contents, respectively, which are quite high in comparison with previously published data. Ahmed et al. [6] studied the TPC and TFC of different solvent extracts of Cannabis. These findings show that methanolic extract had maximum TPC (36.42±1.905 mg GAE/g) and TFC (59.03±1.312 mg QE/g) followed by distilled water and ethanol [6]. The aforementioned findings have shown that the difference in the TPC and TFC content primarily depend on the plant part, solvent used for extraction, different variants of Cannabis and the environmental conditions in which plants were grown.

In GC-MS analysis, we identified about 28 compounds in CEO. The oil composition is slightly different than the previous report by Nissen et al. [31], who found sesquiterpene and monoterpenes as main compounds present in industrial hemp varieties. Our results are also not completely in agreement with the work of Gulluni et al. [32] who identified 35 compounds from hemp inflorescences. The predominant compounds present in the extract were myrcene (22.9%) and β-caryophyllene. However, the results of the present study are in concordance with Qureshi et al. [33] who found tetrahydrocannabinol, cannabidiol and cannabino as major constituents in the Cannabis
cultivars found in Khyber Pakhtunkhwa region of Pakistan [33]. Likewise, GC-MS analysis of *C. sativa* collected from different regions of Pakistan have nearly the same chemical composition as that of our sample e.g. tetrahydrocannabinol, phytol, cannabiol, 9-anthracenecarbonitrile, azapyrene. So, from the aforementioned findings it is clear that the difference in the chemical profile of the extract mainly depends on the region of sample collection, growth conditions, extraction solvent and analytical method used for chemical characterization.

**Conflict of interest:** Authors declare no conflict of interest.

**CONCLUSION**

There is a plethora of data available on phytochemical and pharmacological properties of *Cannabis*, whereas the current study is an effort to advert the attention towards the *Cannabis* species available in Pakistan. In Pakistan, *Cannabis* is found as a wild plant, however, it has also been cultivated in tribal agencies which are part of Northern areas of the country. Moreover, the people who cultivate *Cannabis* have very little knowledge regarding the medicinal potential of the plant; therefore a huge amount of plant material is only being used for recreational purposes. Our data shows that along with antimicrobial activity, CEO also has high TPC and TFC. CEO contains medicinally important chemical compounds, particularly cannabinoids, which are known for their pharmacological potential. Further studies will extend our knowledge about the cannabinoid contents of *Cannabis* varieties available in Pakistan.

**REFERENCES**


16. Chew KK, Khoo MZ, Ng SY, Thoo YY, Aida WW, Ho CW. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Orthosiphon stamineus* extracts. Int Food Res J 2011; 18:1427.


29. Agarwal C, Máté K, Hofmann T, Csóka L. Ultrasound-assisted extraction of cannabinoids from *Cannabis sativa* L. optimized by response surface methodology. J Food Sci 2018; 83:700-


