Formulation of herbal tea using Cymbopogon citratus, Foeniculum vulgare and Murraya koenigii and its anti-obesity potential

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FORMULATION OF HERBAL TEA USING *CYMBOPOGON CITRATUS*, *FOENICULUM VULGARE* AND *MURRAYA KOENIGII* AND ITS ANTI-OBESITY POTENTIAL

Abstract

Objectives: The term 'herbal tea' refers to a beverage made from the medicinal plants, herbs and spices. Herbal teas are non-caffeinated and consumed worldwide due to their therapeutic and healing properties. The present study aimed at the development of herbal tea formulation using *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves and its qualitative and quantitative evaluation.

Methods: Sensory evaluation of tea was carried out using 9point headonic Scale. Qualitative and quantitative phytochemical analysis, antioxidant activity using DPPH assay and antimicrobial activity using disc diffusion method was carried out. In addition to this molecular docking of different chemical compounds present in *Cymbopogon citratus, Foeniculum vulgare* and *Murraya koenigii* was performed against Alpha-ketoglutarate-dependent dioxygenase also called at mass and obesity-associated protein FTO.

Results: Formulation 1 possessed better sensory attributes than other formulations. Physicochemical analysis of extracts indicated the presence of 20-22 % moisture content and 13-18 % ash content in all tested extracts. Phytochemical analysis of extracts indicated the presence of various phytochemicals like alkaloids, tannins, phenols, flavonoids etc. in all tested extracts. Antioxidant potential of extracts increased with the increasing volume of extracts. All tested extracts possessed higher antimicrobial potential against *S. aureus* as compared to *E. coli*. It was found that herbal tea extract possessed highest antimicrobial potential against both tested strains as compared to individual plant extracts. FTIR analysis of extracts indicated the presence of various functional groups in the extracts. *In silico* analysis of compounds indicated that tested compounds possess good binding affinity with FTO protein and show a possible good replacement as synthetic drugs to prevent and treat obesity.

Conclusion: The *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves could become a good source of constituents for formulation of herbal tea which was acceptable to consumers and possess good antimicrobial, antioxidant and anti-obesity potential.

Key Words: Antimicrobial, FTO protein, Herbal tea, Phytochemicals

1. Introduction

Tea is the most extensively consumed beverage on the planet after water. It is commonly prepared by boiling leaves of tea plant (*Camelia sinensis*) in hot water (Alakali *et al.,* 2016). Currently, tea is a hot topic in nutritional and medicinal study all around the world. This is not due to the popularity of tea, but because of the presence of several therapeutic compounds in tea which possess antimicrobial, antioxidant and other biological activities. There are three basic types of tea depending upon the degree of fermentation and various methods of tea plant processing, but all made from the same tea plant (*Camellia sinesis*). The fully fermented tea plant is used to make black tea, oolong tea is made from semi fermented tea plant and non-fermented tea plant is used to make green tea. Phytochemicals present in the leaves of tea plant like polyphenols and flavonoids possess antioxidant activity and other biological activities. Plants synthesize these chemicals as a result of secondary metabolism or in reaction to various microbial infections. These compounds are of great medicinal importance, although they are found only in trace amounts in plant tissues (Bansode, 2015).

Tea use has long been encouraged due to its many health advantages, including a decrease in cardiovascular disorders and various types of cancer. It also increases mineral density of bones and shows neuro-protective and anti-fibrotic properties.Tea is very good for oral health. It reduces blood pressure, helps in controlling body weight and possesses antibacterial activity (Heinrich *et al.,* 2011).

The term ‘herbal tea’ refers to a beverage made from the medicinal plants, herbs and spices and sold in a form of tea bags or in loose form. It produced by brewing from the various plant

parts such as leaves flower fruits, roots, stem and seeds from different plant species other than *Camellia sinesis*. Herbal tea is non-caffeinated and is different from other beverages like coffee and true tea. Due to its medicinal and healing qualities, herbal teas are drunk all over the world (Alakali *et al*., 2016).

Nowadays, there is a huge interest in functional beverages because of rapidly growing awareness among consumers of health benefits derived from the consumption of herbal tea. There are more than 4000 bioactive compounds present in herbal tea, in which polyphenols accounts for one-third ratio and the rest is covered by tannins and flavonoids. Herbs also contain wide variety of antioxidants which are responsible for the neutralization of unwanted free radicals. The antioxidant activity of many plants is thought to be mostly due to phenolic constituents which are present in many fruits, vegetables, and tea (Anand *et al*., 2015).

Cymbopogon citratus (lemon grass) belongs to 'Poaceae' family. It is a tall, aromatic, and perennial plant with green leaves. Name of the plant is derived from its lemon like aroma. Its leaves are used as flavoring agent in major foods like herbal teas, frozen desserts, and meat products. Lemongrass has a wide range of medicinal properties, making it one of the healthiest plants available. Due to its medicinal properties, this herb can be used for treating cold, cough, fever, headache, abdominal pain, stomach-ache, and rheumatic pain. Besides this, lemongrass tea is also known for its antidiuretic, antimicrobial, mood enhancer, anti-inflammatory, antidepressant, and sedative activities (Umar *et al.,* 2016).

Foeniculum vulgare belongs to the 'Apiaceae' family. Fennel seeds are not only delicious in taste but are also health promoting because of their nutritional content. It contains a lot of antioxidants, flavonoids, and carotenoids. Antioxidants are present in very high levels, and they are responsible for the neutralization of free radicals present in the body thus preventing cell damage. Fennel seeds are not only antibacterial in nature, but they are also high in antioxidants (Verma, 2018).

Murraya koenigii, is an aromatic perennial shrub. This tree's leaves are known as 'curry leaves. The leaves have a little pungent, bitter, and acidic flavor. Leaves of *Murraya koenigii*

are reported to contain high amount of carbohydrates, proteins, amino acids, and alkaloids. They are also rich source of vitamin A, vitamin B and minerals. High amount of calcium is also present in curry leaves. They are also rich in crystalline glucoside, resin and koenigiin, Carbazole alkaloids. Traditionally, *Murraya koenigii* is used as an antiemetic, antidiarrheal, antibacterial, antioxidant, blood purifier, antifungal, antiulcer, anticancer, antidiabetic, antidepressant, anti-inflammatory and for relieving body aches and kidney pain (Husain and Trak, 2018).

Obesity has recently emerged as a global health issue leading to a variety of health issues like hyperlipidemia, cardiovascular diseases, type II diabetes and different forms of cancer. Synthetic drugs that are used for treating obesity have a number of side effects, so there is a need to search for alternative therapies. Molecular docking is a bioinformatics technique which is used for the determination of binding efficiency or energy of different compounds or phytoconstituents with the targeted protein and helps to identify the specific biological response it elicits in the body. The FTO (fat mass and obesity-associated protein) is a dioxygenase enzyme that acts on N6-methyladenosine and N6, 2′-O-dimethyladenosine in mRNA of eukaryotes to cause an internal alteration (Ruud *et al*., 2019). Several studies confirmed the link between FTO gene polymorphism and increased body mass. Phytoconstituents present in various plants can help in preventing or reducing obesity by targeting FTO protein (Church *et al*., 2009).

Herbal teas have therapeutic and immune-boosting effects, making them a viable alternative to the conventional medicine. So present study aimed at the development of herbal tea formulation using *Cymbopogon citratus* (lemon grass), *Foeniculum vulgare* (fennel) and *Murraya koenigii* (curry leaves) and evaluate their combined effect.

2. Materials and methods

2.1. Sample preparation

Cymbopogon citratus and *Murraya koenigii* leaves were shade dried and then ground to make powder. The seeds of *Foeniculum vulgare* seeds were processed via an electric

grinder into a fine powder. All powders were stored in glass containers separately (Saleem *et al*., 2019).

2.2. Extract preparation

One gram of dried herbs powder was soaked in 50 ml of hot water at 75 - 96 °C for 2 - 5 minutes and filtered after wards (Ueda *et al*., 2019).

2.3. Formulation of herbal tea

Herbal tea was made by combining powdered *Cymbopogon citratus* leaves, *Murraya koenigii* leaves and *Foeniculum vulgare* seeds in different proportions (TableS1).

2.4. Sensory evaluation

Sensory evaluation of herbal tea formulations was conducted using 9-point hedonic scale (Saleem *et al*., 2019).

2.5 Physicochemical analysis

Physicochemical analysis of each sample extract including moisture and ash contents, pH and total soluble solids were determined (AOAC, 2005).

2.6. Antioxidant analysis

Antioxidant activity of each sample extract was evaluated according to Brand-Williams *et al*. (1995). The following formula was used to determine a DPPH radical scavenging potential and express it as a percentage of scavenging potential.

Radical scavenging activity (%) = (OD Control – OD Sample / OD Control) × 100

2.7. Determination of antimicrobial activity

Antimicrobial activity of each extract was determined against *E. coli* and *S. aureus* (Rashmi and Naveen, 2016).

2.8. Qualitative phytochemical analysis

Qualitative analysis of different phytochemicals including phylobatannins, tannins, anthraquinones, carotenoid, terpenoids, flavonoids, steroids, saponins, proteins, carbohydrates, quinones, coumarins, alkaloids, glycosides, phytosterol and phenolic compounds present in herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves extracts was performed (Soni and Sosa, 2013;; Shaikh and Patil, 2020).

2.9. *Quantitative phytochemical analysis*

2.9.1. *Determination of total phenolic content*

Total phenolic content of each sample extract was determined using Gallic acid as a standard. 250 µl of Folin - Ciocalteu reagent was taken and added in 250 µl of each sample extract. The mixture was shaken thoroughly and left at room temperature for 5 minutes. This step allowed the complete reaction to occur with the Folin-Ciocalteu reagent. After the addition of 2.5 ml of 7 % Sodium carbonate solution, the final volume was raised up to 10 ml with distilled water. Test tubes were incubated for 90 minutes at room temperature. The absorbance of the solution was then taken at 765 nm using spectrophotometer (Soni and Sosa, 2013).

2.9.2. *Determination of alkaloids*

Alkaloid content of herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves was determined according to Soni and Sosa (2013). In 5 g powder of each sample 200 ml of 10 % acetic acid solution was added. The solution was allowed to rest for 4 hours. Following that, the solution was filtered and concentrated over a water bath until it was one-quarter of its original volume. Ammonium hydroxide was then added drop wise to the solution until precipitation. Precipitates were allowed to settle and then collected. Percentage of alkaloid was calculated using following formula,

% Alkaloid = Weight of Alkaloid / Weight of Sample × 100

2.9.3. *Determination of flavonoids*

Flavonoid content of each sample extract was determined according to Akande *et al*. (2011). 0.5 ml of each sample extract was added in 1 ml of distilled water. The solution was then mixed with 75 µl of 5 % Sodium nitrite. 75 µl of 10 % Aluminium chloride was mixed after 5 minutes. The mixture was left for 5 minutes and then 0.5 ml of 1M NaOH was added. The mixture was shaken thoroughly and kept for 15 minutes. Absorbance of sample was then taken at 510 nm using spectrophotometer. Flavonoid content of each sample extract was then determined using quercetin standard curve.

2.9.4. Determination of tannins

0.1 ml of each sample extract was added to the 8 ml of distilled water. 0.5 ml of Folin-Ciocalteu reagent and 1 ml of 35 % Na_2CO_3 solution were then added. The mixture was shaken well and kept in dark for 30 minutes at room temperature. Absorbance of sample was taken at 725nm with a spectrophotometer. Tannin content of each sample extract was determined using tannic acid standard curve (Mythili *et al*., 2014).

2.10. Statistical analysis

The post-hoc multiple comparison test under a one-way ANOVA was carried out using SPSS on all of the experimental data. Significance has been presented at the level of ≤ 0.05.

2.11. FTIR analysis

FTIR analysis of herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves was conducted using IRTracer-100 FTIR Spectrophotometer for the identification of different functional groups present in the samples (Nandiyanto *et al*., 2019).

2.12. In silico analysis of herbal compounds

Molecular docking of different chemical compounds present in *Cymbopogon citratus*, *Foeniculum vulgare* and *Murraya koenigii* was performed against FTO protein (accession no. Q9C0B1) using GalaxyWeb (Singh *et al*., 2021).The different compound determined by FTIR were retrieved from Pubchem and used for docking with FTO protein (Kim *et al*., 2023).

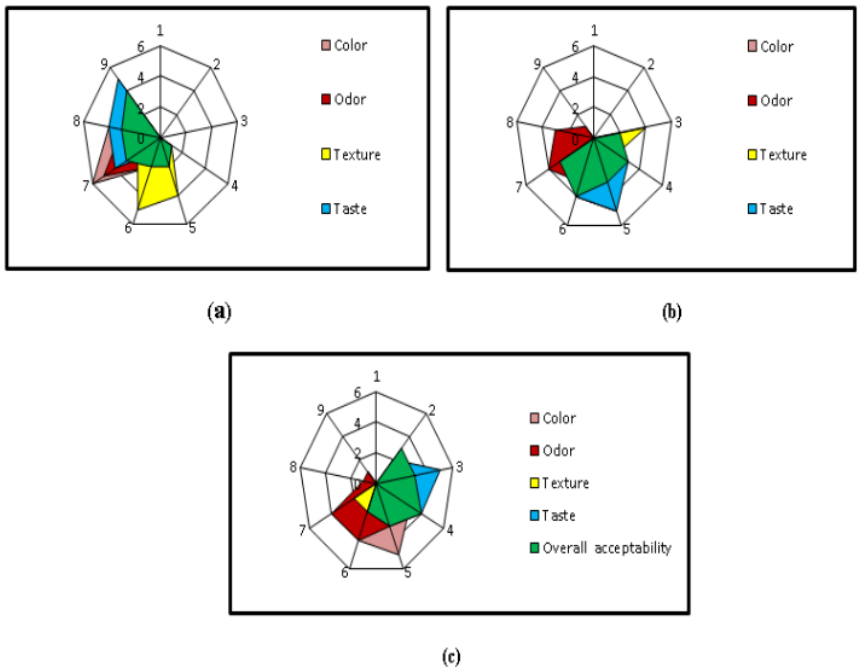
3. Results

3.1. Sensory evaluation

In sensory evaluation, different sensory properties like color, taste, aroma and texture were evaluated by judges. Significant changes were observed in sensory attributes of different formulations. 'Like extremely' to 'like moderately' was considered as positive or 'liked', 'like slightly' to 'dislike slightly' was set as neutral and 'dislike moderately' to 'dislike extremely' was considered as negative or 'disliked' part of the scale (Fig. 1). Formulation 1 was found to be overall acceptable by the judges as compared to other formulations. It possessed better sensory attributes as compared to other formulations (Fig. 1).

3.2. Physicochemical analysis

In physicochemical analysis, results indicated the presence of 20 %, 20.5 %, 22 % and 19.5 % moisture content in herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves extracts respectively (Fig. 2 a). Highest moisture content was possessed by *Foeniculum vulgare* seeds while the lowest moisture content was possessed by *Murraya koenigii* leaves. Herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves possessed ash content of 18 %, 15.6 %, 13 % and 16 %, respectively (Fig. 2b). Highest percentage of ash was possessed by herbal tea extract and the lowest ash percentage was possessed by *Foeniculum vulgare* seeds. All samples showed pH values close to neutral (Fig. 2c). Both herbal tea extract and *Murraya koenigii* leaves extract possessed 1 ℬ total soluble solids (Fig. 2d).



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203 **Fig. 1. Sensory evaluation of herbal tea formulations (a) formulation 1 (b) formulation 2**

204 **(c) formulation 3**

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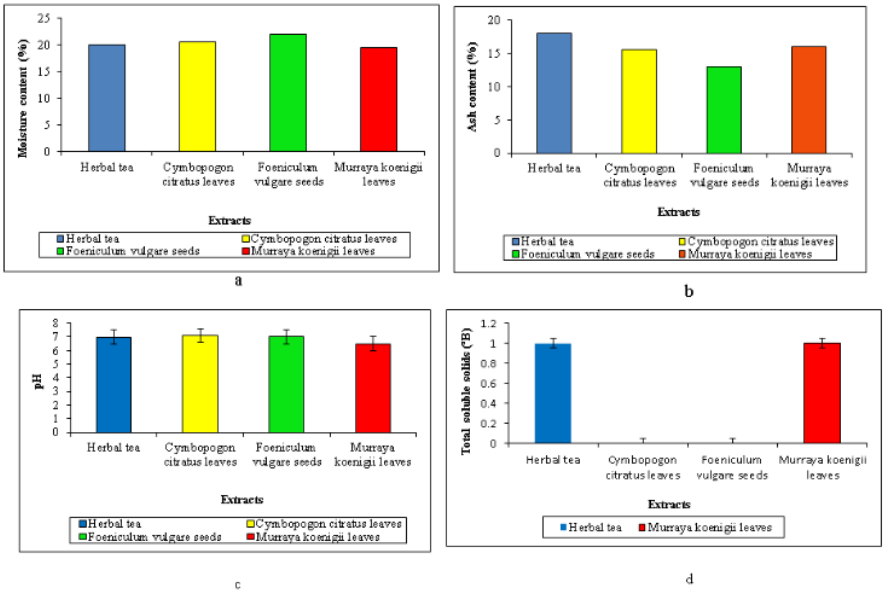

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212 **Fig. 2. Determination of herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare***
213 **seeds and *Murraya koenigii* leaves extracts (a) Moisture contents (b) Ash contents (c) pH**
214 **(d) Total soluble Solids**

215 *3.3. Qualitative phytochemical analysis*

216 Qualitative phytochemical analysis indicated the presence of tannins, flavonoids, saponins,
217 quinones, coumarins, alkaloids, glycosides and phenolic compounds in all extracts (TableS2).
218 Presence of carbohydrates and terpenoids was shown by extracts of herbal tea and *Murraya*
219 *koenigii* leaves. *Foeniculum vulgare* seeds extract indicated the presence of proteins. Presence
220 of phytochemicals like phytosterol, phylobatannins, anthraquinones, carotenoids and steroids
221 was not indicted by any tested extract.

222 *3.4. Quantitative phytochemical analysis*

223 Quantitative phytochemical analysis indicated that tannin contents of 2.752, 0.589, 1.057,
224 1.195 (mg TAE/ml) and total phenolic content of 0.241, 0.115, 0.015, 1.06 (mg GAE/ml)
225 were possessed by herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and
226 *Murraya koenigii* leaves extracts, respectively (Fig. 3b & c). Flavonoid contents of 1.639,

2.253, 1.001, 2.958 (mg QE/ml) were possessed by herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves extracts, respectively (Fig. 3d). Highest percentage of alkaloid was indicated by *Foeniculum vulgare* seeds extract (Fig. 3a).

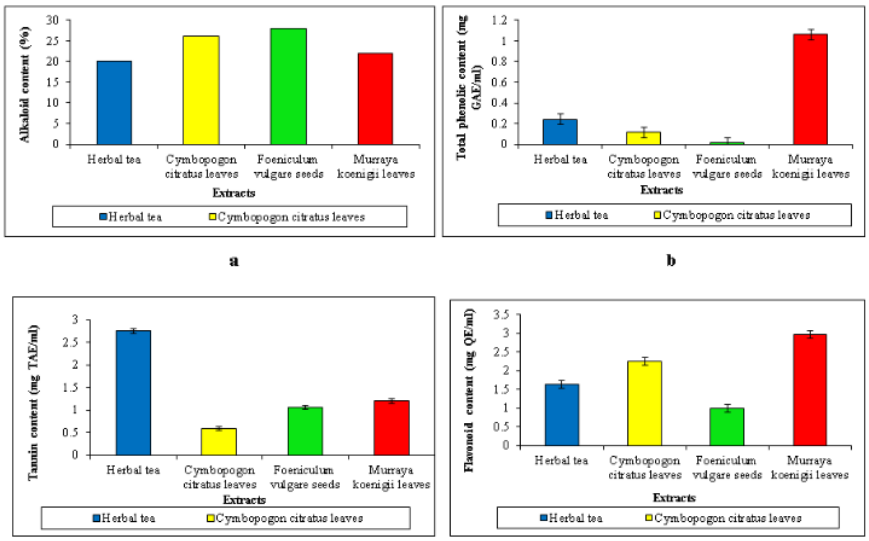

Fig. 3. Determination in aqueous extracts of herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves (a) Alkaloid contents (b) Total phenolic contents (c) Tannin contents (d) Flavonoids contents

3.5. Antioxidant activity

All tested plant extracts including herbal tea possessed significant antioxidant potential. Highest antioxidant potential was shown by herbal tea extract as compared to other extracts (Figure 4a). Results of the study indicated that antioxidant potential of extracts increased with the increasing volume of extracts. Highest antioxidant activity was possessed by herbal tea using 200 μl of extract while the lowest activity was shown by *Foeniculum vulgare* seeds using 100 μl extract (Fig. 4a).

3.6. Antimicrobial potential

All tested extracts possessed higher antimicrobial potential against *S. aureus* as compared to *E. coli*. *Foeniculum vulgare* seeds extract possessed no activity against *E. coli* (Fig. 4b). Results revealed that herbal tea extract showed highest antimicrobial potential against both tested strains as compared to individual plant extracts. Maximum inhibition zone of 13.5 mm was shown by herbal tea extract against *S. aureus* and the minimum inhibition zone of 6 mm was shown by *Murraya koenigii* leaves extract against *E. coli* (Fig. 4b & c).

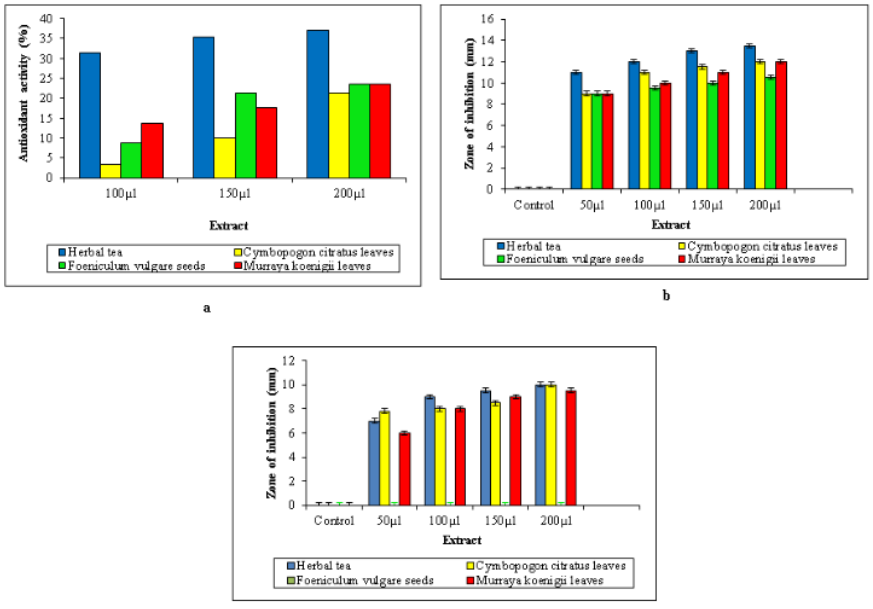

Fig. 4. Determination in aqueous extracts of herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves (a) antioxidant activity (b) antimicrobial activity against *S. aureus* (c) antimicrobial activity against *E. coli*

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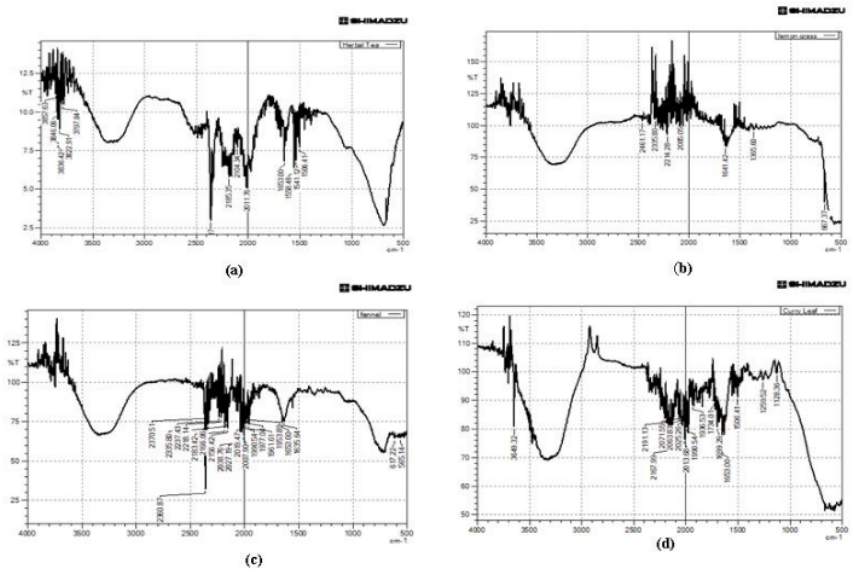
259 *3.7. FTIR analysis*

260 FTIR spectrum of herbal tea, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and
261 *Murraya koenigii* leaves extracts indicated the presence of various peaks corresponding to
262 different functional groups in the extracts (Fig. 5).



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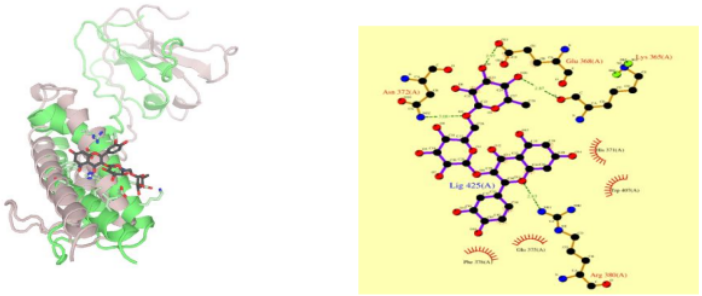
264 **Fig. 5. FTIR analysis of extracts (a) herbal tea (b) *Cymbopogon citratus leaves* (c)**
265 ***Foeniculum vulgare seeds* (d) *Murraya koenigii leaves***

266 *3.8. Binding of ligands to FTO protein*

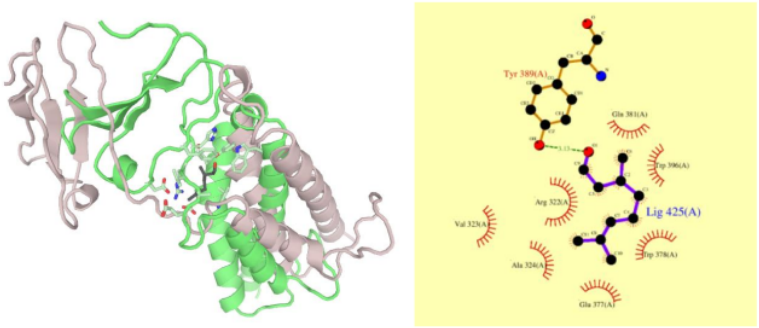
267 Interactions of rutin, citronellal and vanillic acid present in *Foeniculum vulgare* seeds,
268 *Cymbopogon citratus* leaves and *Murraya koenigii* leaves respectively, with binding site of
269 FTO protein were shown in Figure 6-8 and TableS3. Ligands were bound with the active site
270 of the FTO protein. Different hydrogen bonds and hydrophobic interactions were formed
271 between ligands structure and protein (Fig. 6-8). It was observed that 4 hydrogen bonds, 1
272 and no hydrogen bond were formed with rutin, citronellal and vanillic acid respectively (Fig.

6-8). These different ligands were retrieved from the Pubchem database (Kim *et al*., 2023) and were used for docking with the FTO protein.



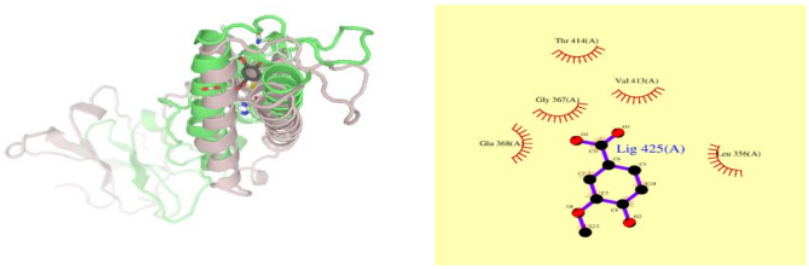
(a) (b)

Fig. 6: Molecular docking of rutin to FTO protein (a) 3D structure of interaction of rutin with FTO protein binding site (b) Residues in contact of protein FTO with rutin



(a) (b)

Fig. 7. Molecular docking of citronellal to FTO protein (a) 3D structure of interaction of citronellal with FTO protein binding site (b) Residues in contact of protein FTO with citronellal



(a) **(b)**

Fig. 8. Molecular docking of vanillic acid to FTO protein (a) 3D structure of interaction of vanillic acid with FTO protein binding site (b) Residues in contact of protein FTO with vanillic acid

4. Discussion

Herbal teas possess therapeutic and immune-boosting effects, making them a viable alternative to the conventional medicine. In present study sensory evaluation of herbal tea formulations indicated that formulation 1 received highest sensory scores for parameters like color, aroma, taste and texture. This could be attributed to the presence of the highest percentage of *Cymbopogon citratus* leaves in formulation 1, which had a pleasant taste and aroma. These parameters are very important in determining the quality of herbal tea. Color is a sensation that is part of the visual sense and is used to judge the appearance of a food product. Similarly, aroma is a characteristic of tea quality that can determine whether a tea is accepted or rejected before it is tasted. Least sensory score for taste was obtained for formulation 3. This could be due to the addition of larger amount of *Murraya koenigii* leaves in formulation 3 which possessed highest amount of tannins resulting in the bitter taste of tea.

So, formulation 1 was found to be overall acceptable by the judges. Moisture and ash contents are the important factors that determine the quality of products during storage. Results of the study indicated that moisture content of all extracts was in the range of 20 to 22 %. Our results are in agreement with the findings of Ahmadi *et al*. (2009) who reported the similar value of moisture content (21 %) in *Foeniculum vulgare* seeds. The low moisture content indicated that all dried plant samples were likely to last longer before being used or processed, as the low moisture content of the leaves combined with drying could inhibit microbial growth, resulting in a longer storage life (Awogbemi and Ogunleye, 2009). Results of the study indicated that ash content of all extracts was in the range of 13 to 18 %. Our results concur with those of Igara *et al*. (2016) who reported the similar ash content value (15.07 %) in *Murraya koenigii* leaves. Results of the study are contradictory to Varma and Parnami (2019) who reported the different ash content value (9.68 %) in dried *Murraya koenigii* leaves. Different ash content values of samples might be attributed to the differences in the drying conditions. Higher ash content values are associated with lower moisture content as a result of drying (Mabai *et al.,* 2018). In current study pH of all sample extracts was observed to be close to neutral. pH values of all extracts indicated that all tested plants could be safely employed in the formulation of herbal tea. Our findings are consistent with those of Mabai *et al*. (2018), who showed that the leaves of *Cymbopogon citratus* have a comparable pH value. The variation in pH values of samples might be due to the differences in the drying air temperature, relative humidity of the drying air, nature of the drying air flow and air exposure time (Mabai *et al.,* 2018). TSS corresponds to the amount of total soluble sugars present in a liquid sample that can affect the quality and taste of the sample. Results of the present study indicated the presence of total soluble solids in *Murraya koenigii* leaves extract and herbal tea extract. Qualitative phytochemical analysis indicated the presence of various phytochemicals like tannins, flavonoids, alkaloids and phenolic compound in all tested extracts. Presence of carbohydrates and terpenoids was shown by extracts of herbal tea and *Murraya koenigii* leaves. This might be due to the addition of *Murraya koenigii* leaves in herbal tea formulation because both other ingredients did not possess these phytochemicals

(Alzobaay and Kadhim, 2018). *Foeniculum vulgare* seeds extract indicated the presence of proteins. Results of the study are compatible with Chatterjee *et al*. (2012) and Deepika and Noorjahan (2016) who studied the phytochemical profiles of *Murraya koenigii* leaves and *Foeniculum vulgare* seeds and reported the presence of similar phytochemical compounds in the extracts. Results of the study are also in agreement with the findings of Unuigbe *et al*. (2019) who reported the presence of similar phytochemicals in *Cymbopogon citratus* leaves extract. Quantitative phytochemical analysis indicated that highest values of phytochemicals were observed for *Murraya koenigii* leaves extract. Highest percentage of alkaloid was indicated by *Foeniculum vulgare* seeds extract. Our study is compatible with the findings of Tangkanakul *et al*. (2009) who reported similar values of total phenolic content in *Cymbopogon citratus* leaves and *Murraya koenigii* leaves. Results of our study are also compatible with the findings of Rajic *et al*. (2018) who reported similar values of total flavonoid and phenolic content in *Foeniculum vulgare* seeds. However, our results are contradictory to Thorat *et al*. (2017) who reported different values of total phenolic, flavonoid and tannin content in *Cymbopogon citratus* leaves. Different phytochemical values of plant samples might be attributed to variety of plant, growing environment and particle size of extract powder (Astill *et al*., 2001). The variation in total flavonoid content of samples could be attributed to a variety of factors such as agronomic conditions, processing methods, storage during and after transport, as well as the degree of fermentation (Bansode, 2015). Results of antioxidant analysis of sample extracts indicated the increase in antioxidant potential with the increasing concentration of extracts. This might be due to the presence of large number of antioxidant compounds in the higher concentrations of the extracts. Results of the study indicated that herbal tea extract possessed higher antioxidant potential at all concentrations as compared to other extracts. This could be attributed to the combination of plants used in formulation of herbal tea which in turn led to higher concentration of antioxidant compounds like polyphenols in herbal tea. Our results are supported by the findings of Radali and Alka (2018) who also reported the similar value of antioxidant potential in *Cymbopogon citratus* leaves extract. Results of antimicrobial analysis indicated

that higher antimicrobial potential was possessed by herbal tea extract against both tested strains as compared to the individual plant extracts. This could be attributed to the combination of plants used in formulation of herbal tea which in turn led to higher concentration of phytochemical compounds in herbal tea. Results also revealed that antimicrobial potential of all extracts increased with the increasing concentrations of the extracts used. This might be due to the presence of large number of extracted compounds in higher concentrations of extracts (Balakrishnan *et al*., 2014). Antimicrobial activities of the extracts could be attributed to the presence of different phytochemical compounds like flavonoids and polyphenols present in the extracts All tested extracts possessed higher antimicrobial potential against Gram positive bacterial strain as compared to Gram negative bacterial strain. This might be due to the presence of differences in cell wall structures and bacterial complexities (Ewansiha *et al*., 2012). Our results are in agreement with the findings of Balakrishnan *et al*. (2014) who reported similar antimicrobial potential of aqueous *Cymbopogon citratus* leaves extracts against *S. aureus*. Results of the present study are also in accordance with the findings of Benlafya *et al*. (2015) who reported that no antibacterial activity was shown by aqueous *Foeniculum vulgare* seeds extract against *E. coli*.. The Results of the FTIR analysis of extracts indicated the presence of organic and aromatic compounds that might be responsible for the antimicrobial, antioxidant and other biological activities of the extracts. Several studies justify our results of FTIR analysis. Oyenike *et al*. (2018) reported the presence of similar functional groups like alcohol, carbonyl group, carboxyl group, phenol using FTIR analysis in *Cymbopogon citratus* leaves extract. In the present study it has been investigated the anti-obesity potential of certain phytoconstituents by targeting FTO protein. The FTO (fat mass and obesity-associated protein) is a dioxygenase enzyme that acts on N6-methyladenosine and N6, 2′-O-dimethyladenosine in mRNA of eukaryotes to cause an internal alteration (Ruud *et al*., 2019). Several studies have confirmed the link between FTO gene polymorphism and increased body mass (Church *et al*., 2009). Ligands that were selected for docking studies comprised of Rutin (a glycoside made up of the flavonol quercetin and rutinose), Citronellal (a monoterpenoid aldehyde) and Vanillic acid

(a derivative of dihydroxybenzoic acid). Ligands were selected after comparing their FTIR peaks from PubChem with the FTIR spectra of tested plants. Results of the study showed that rutin, a flavonoid formed more hydrogen bonds as compared to citronellal and vanillic acid. Similarly, rutin showed the highest binding affinity (most negative docking energy value) to FTO protein. It was found that there were 4 hydrogen bonds, 1 and no hydrogen bond in rutin, citronellal and vanillic acid respectively. Anti-obesity potential of the tested ligands was in an order of rutin > citronellal > vanillic acid. This might be due to the higher lipolytic activity of rutin (Kuppusamy and Das, 1992). The binding affinity of the tested ligands with the FTO protein for acting as an inhibitor was found to be higher as compared to certain flavonoids reported in previous research. Similarly, docking results of tested ligands were found to be better as compared to drug (Orlistat) as reported by Mohammed *et al*. (2015). The idea that a SNP in the mouse FTO protein resulted in a slender type mouse suggested a link between FTO demethylase activity and fat mass (Church *et al.,* 2009). In rat adipose cells, a variety of flavonoids were reported to possess lipolytic properties. Phytochemical compounds affect lipolysis and adipogenesis in adipocytes. They suppress the expression of transcription factors linked to adipogenesis (Kuppusamy and Das, 1992).

Conclusion

In present study herbal tea was formulated using *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves and it's qualitative and quantitative analysis was conducted. It was found that good antimicrobial and antioxidant potential was possessed by herbal tea which was also appealing to consumers. Several important phytochemicals were possessed by herbal tea responsible for its biological properties. FTIR analysis indicated the presence of various functional groups in herbal tea. It was found that tested compounds present in herbal tea possessed good binding affinity to target protein and could replace synthetic drugs to prevent and treat obesity. So, *Cymbopogon citratus* leaves, *Foeniculum vulgare* seeds and *Murraya koenigii* leaves could be a good source of constituents for

formulation of herbal tea which was acceptable to consumers and possessed good antimicrobial, antioxidant and anti-obesity potential.

References

Ahmadi, H., Mollazade, K., Khorshidi, J., Mohtasebi, S. S. and Rajabipour, A., 2009. Some physical and mechanical properties of fennel seed (*Foeniculum vulgare*). J.Agric. Sci. 1, 66-75. DOI: 10.5539/jas.v1n1p66

Akande, I. S., Samuel, T. A., Agbazue, U., Olowolagba, B. L. 2011. Comparative proximate analysis of ethanolic and water extracts of *Cymbopogon citratus* (lemongrass) and four tea brands. Plant Sci. Res. 3, 29-35. http://dx.doi.org/10.3923/psres.2011.29.35

Alakali, J. S., Ismaila, A. R., Alaka, I. C., Faasema, J., Yaji, T. A., 2016. Quality evaluation of herbal tea blends from ginger and *Pavetta crassipes*. Eur. J. Med. Plants. 12, 1-8. http://dx.doi.org/10.9734/EJMP/2016/23706

Alzobaay, A. H., Kadhim, B. H., 2018. Phytochemical screening, chemical composition and antibacterial activity of lemongrass (*Cymbopogon citratus*) leave extracts. Ind. J. Nat. Sci. 9, 15306-15315.

Anand, J., Upadhyaya, B., Rawat, P., Rai, N., 2015. Biochemical characterization and pharmacognostic evaluation of purified catechins in green tea (*Camellia sinensis*) cultivars of India. 3 Biotech, 5, 285-294. https://doi.org/10.1007%2Fs13205-014-0230-0

AOAC. 2005. *Association of Official Analytical Chemist's,* 18th ed. Washington DC, UAS. pp. 951-952.

Astill, C., Birch, M. R., Dacombe, C., Humphrey, P. G. and Martin, P. T. 2001. Factors affecting the caffeine and polyphenol contents of black and green tea infusions. J. Agric. Food Chem., 49, 5340-5347. https://doi.org/10.1021/jf010759

Awogbeni, O. and Ogunleye, I. O. 2009. Some selected vegetables. Int. J. Eng. Technol. 1, 1793-8236.

Balakrishnan, B., Paramasivam, S., Arulkumar, A., 2014. Evaluation of the lemongrass plant (*Cymbopogon citratus*) extracted in different solvents for antioxidant and

446 antibacterial activity against human pathogens. Asian Pac. J. Trop Dis. 4, 134-139.
447 https://doi.org/10.1016/S2222-1808(14)60428-X
448 Bansode, P. A., 2015. Total flavonoid content of commonly consumed teas in India.
449 World J. Pharm Res. 4, 874-81.
450 Benlafya, K., El Farsaoui, M., Chatouani, S., Azamouze, W., Charkaoui, Y., Karrouchi,
451 K. 2015. Antimicrobial potentials of aqueous and methanolic crude extracts of *Zingiber*
452 *officinale* and *Foeniculum vulgare*. J. Chem. Pharm. Res. 7, 964-966.
453 Brand-Williams, W., Cuvelier, M. E., Berset, C. L. W. T., 1995. Use of a free radical
454 method to evaluate antioxidant activity. LWT-Food Sci. Technol. 28, 25-30.
455 https://doi.org/10.1016/S0023-6438(95)80008-5
456 Chatterjee, S., Goswami, N., Bhatnagar, P., 2012. Estimation of phenolic components and
457 *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) and ajwain (*Trachyspermum*
458 *ammi*) seeds. Adv. Biores. 3, 109-18.
459 Church, C., Lee, S., Bagg, E. A., Mctaggart, J. S., Deacon, R., Gerken, T., Cox, R. D.,
460 2009. A mouse model for the metabolic effects of the human fat mass and obesity
461 associated FTO gene. Plos Genet. 5, e1000599.
462 https://doi.org/10.1371/journal.pgen.1000599.
463 Deepika, T., Noorjahan, C. M., 2016. Phytochemical screening and thin layer
464 chromatographic analysis for antioxidant activity of *Murraya koenigii* (Curry leaf). Int. J.
465 Pharm. Life Sci. 7, 5369-5374.
466 Ewansiha, J. U., Garba, S. A., Mawak, J. D., Oyewole, O. A., 2012. Antimicrobial
467 activity of *Cymbopogon citratus* (Lemon grass) and its phytochemical properties. Front
468 Sci, 2, 214-220. DOI: 10.5923/j.fs.20120206.l4
469 Heinrich, U., Moore, C. E., De Spirt, S., Tronnier, H., Stahl, W., 2011. Green tea
470 polyphenols provide photoprotection, increase microcirculation, and modulate skin
471 properties of women. J. Nutr. 141, 1202-1208. https://doi.org/10.3945/jn.110.136465

472 Husain, N., Trak, T. H., 2018. Green Herbs as natural healers. World J. Pharm. Res. 7,
473 558-565. DOI: 10.20959/wjpr20185-11338
474 Igara, C. E., Omoboyowa, D. A., Ahuchaogu, A. A., Orji, N. U., Ndukwe, M. K., 2016.
475 Phytochemical and nutritional profile of *Murraya koenigii* (Linn) Spreng leaf. J.
476 Pharmacogn Phytochem, 5. 7-9.
477 Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., Li, Q., Shoemaker, B.A.,
478 Thiessen, P.A., Yu, B., Zaslavsky, L., Zhang, J., Bolton, E.E. 2023. PubChem 2023
479 update. Nucl. Acids Res. 6(D1): D1373-D1380. doi:10.1093/nar/gkac956.
480 Kuppusamy, U. R., Das, N. P. 1992. Effects of flavonoids on cyclic AMP
481 phosphodiesterase and lipid mobilization in rat adipocytes. Biochem. Pharmacol. 44,
482 1307-1315. DOI: 10.1016/0006-2952(92)90531-m
483 Mabai, P., Omolola, A., Jideani, A. I. 2018. Effect of drying on quality and sensory
484 attributes of lemongrass (*Cymbopogon citratus*) tea. J. Food Res. 7, 68-76.
485 https://doi.org/10.5539/jfr.v7n2p68
486 Mohammed, A., Al-Numair, K. S., Balakrishnan, A., 2015. Docking studies on the
487 interaction of flavonoids with fat mass and obesity associated protein. Pak. J. Pharm. Sci.
488 28, 1647-1653. PMID: 26408884
489 Mythili, K., Reddy, C. U., Chamundeeswari, D., Manna, P. K., 2014. Determination of
490 total phenol, alkaloid, flavonoid, and tannin in different extracts of *Calanthe triplicata*. J.
491 Pharmacogn. Phytochem. 2, 40-44.
492 Nandiyanto, A. B. D., Oktiani, R., Ragadhita, R., 2019. How to read and interpret FTIR
493 spectroscope of organic material. Indones. J. Sci. Technol. 4, 97-118.
494 Oyenike, O. A., Osibote, E., Adedugba, A., Bhadmus, O. A., Adeoshun, A. A., Allison,
495 M. O., 2018. Antioxidant activity, total phenolic contents and functional group
496 identification of leaf extracts among lemongrass (*Cymbopogon citratus*) accessions.
497 Nigeria Soc. Exp. Biol. J. 18, 83-91. https://ir.unilag.edu.ng/handle/123456789/5087

498 Radali, D., Alka, G., 2018. Periwinkle (*Catharanthus roseus*) leaves and lemongrass
499 (*Cympoogon citratus*): An analysis of their nutritional composition, anti-nutritional
500 factors and antioxidant. content. Int. J. Curr. Microbiol. Appl. Sci. 7, 2130-2135.
501 http://dx.doi.org/10.20546/ijcmas.2018.706.253
502 Rajic, J. R., Dordevic, S. M., Tesevic, V., Zivkovic, M. B., Dordevic, N. O., Paunovic, D.
503 M., Petrovic, T. S., 2018. The extract of fennel fruit as a potential natural additive in food
504 industry. J. Agric. Sci. 63, 205-215. http://dx.doi.org/10.2298/JAS1802205R
505 Rashmi, J. B and Naveen, G. 2016. Phytochemical analysis and antibacterial
506 activity of different leaf extracts of *Murraya koenigii*. Int. J. Biochem.
507 Biomolecules, 2, 1-5.
508 Ruud, J., Alber, J., Tokarska, A., Ruud, L. E., Nolte, H., Biglari, N., Bruning, J. C., 2019.
509 The fat mass and obesity-associated protein (FTO) regulates locomotor responses to
510 novelty via D2R medium spiny neurons. Cell Rep. 27, 3182-3198.
511 https://doi.org/10.1016/j.celrep.2019.05.037
512 Saleem, A., Durrani, A. I., Awan, F. B., Irfan, A., Noreen, M., Kamran, M. A., Arif, D.,
513 2019. Preparation of marketable functional food to control hypertension using basil
514 (*Ocimum basillium*) and peppermint (*Mentha piperita*). Int. J. Innov. Sci. Technol. 1, 15-
515 32. https://doi.org/10.33411/IJIST/2019010102
516 Shaikh, J. R., Patil, M., 2020. Qualitative tests for preliminary phytochemical screening:
517 An overview. Int. J. Chem. Stud. 8, 603-608. DOI: 10.22271/chemi.2020.v8.i2i.8834
518 Singh, A. K., Gariya, H. S., Bhatt, A. 2021. *In silico* computational docking study of
519 curry leaves biomolecules (*Murraya koenigii*): A potent anticancerous agent. J. Emerg.
520 Technol. Innov. Res. 8, 346-365.
521 Soni, A., Sosa, S. 2013. Phytochemical analysis and free radical scavenging potential of
522 herbal and medicinal plant extracts. J. Pharmacogn. Phytochem, 2, 22-29.

523 Tangkanakul, P., Auttaviboonkul, P., Niyomwit, B., Lowvitoon, N., Charoenthamawat,
524 P., Trakoontivakorn, G. 2009. Antioxidant capacity, total phenolic content and nutritional
525 composition of Asian foods after thermal processing. Int. Food Res. J. 16, 571-580.
526 Thorat, P. P., Sawte, A. R., Patil, B. M., Kshirsagar, R. B. 2017. Proximate and
527 phytonutrient content of *Cymbopogon citratus* (Lemongrass) leaf extract and preparation
528 of herbal cookies. Int. J. Chem. Stud. 5, 758-762.
529 Ueda, Y., Apiphuwasukcharoen, N., Tsutsumi, S., Matsuda, Y., Areekul, V. Yasuda, S.,
530 2019. Optimization of hot-water extraction of dried yacon herbal tea leaves: enhanced
531 antioxidant activities and total phenolic content by response surface methodology. Food
532 Sci. Technol. Res. 25, 131-139. http://dx.doi.org/10.3136/fstr.25.131
533 Umar, M., Mohammed, I. B., Oko, J. O., Tafinta, I. Y., Aliko, A. A., Jobbi, D. Y., 2016.
534 Phytochemical analysis and antimicrobial effect of lemon grass (*Cymbopogon citratus*)
535 obtained from Zaria, Kaduna State, Nigeria. J. Complement. Altern Med. Res. 1, 1-8.
536 http://dx.doi.org/10.9734/JOCAMR/2016/26783
537 Unuigbe, C., Enahoro, J., Erharuyi, O., Okeri, H. A., 2019. Phytochemical analysis and
538 antioxidant evaluation of lemon grass (*Cymbopogon citratus* DC.) stapf leaves. J. Appl.
539 Sci. Environ Manag, 23, 223-228. https://doi.org/10.4314/jasem.v23i2.4
540 Varma, K. and Parnami, M. 2019. Nutritional composition of dried curry leaf powder
541 (*Murraya koenigii*). J. Emerg. Technol. Innov. Res. 6, 409-412.
542 https://www.jetir.org/papers/JETIR1908C51.pdf

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