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Optimization of Natural Food Preservatives Extraction from Spices and Herbs

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ABSTRACT

Bio preservatives is a natural compound deprived from plants used as a natural food preservation to preserve food and extend their shelf life. The extraction optimization and chemical composition of clove, cinnamon, thyme and fennel were investigated as a natural preservative materials. The extraction optimization conditions were investigated by using different ratio of sample to solvent as follow (1:5, 1:10, 1:15 and 1:20 w/v) at different stirring period (1, 2, 3 and 4 hours) and at different concentrations of ethanol in water (80, 60, 40, 20 and 0%) were determined. Moreover, Phytochemicals constitutes of different studied plants including; total soluble polyphenols; flavonoids, alkaloids and saponin were determined in their alcoholic extracts (80%). The data shown that: ethanol in general, gave the highest total soluble solid (TSS) recovery than water. Moreover, 80% ethanol was the most effective in TSS recovery during the extraction process than the other concentrations for clove, cinnamon and thyme. The TSS value of clove, cinnamon and thyme (recovered by 80% ethanol) were 18.2%, 16.72% and 16.33 % plant, respectively. However, fennel water extract conducted 9.17% when compared with 80% ethanol extract which amounted 9%. The chemical composition of secondary metabolites phytochemicals namely phenolic compounds, saponine, flavonoids and alkaloids which expected to have antibacterial activity, were determined in clove, cinnamon and thyme plants. Data show that no significant difference in phenolic contents between clove and cinnamon. While thyme contained the lowest amounts of phenolic compounds. On the other hand, thyme contained the significant highest amounts (2.63 mg/g dry weight) of alkaloids among the other studied plants.

1. Introduction

Food additives are a chemicals substances that are added to food for a specific purpose in that food such as antimicrobial, antioxidant, anti-browning, anti-staling and vitamins. antimicrobial products are chemicals used to mitigate microbiological organisms on processed food by adding such substances to the processed food as food additive (Branter and Grevn, 1999, Amit et al., 2017). Therefore, the need of naturally food additives is a goal of the different recent research.

Food Bio preservatives are a natural antimicrobials are desirable plant's extracts or volatile oils which they are usually added to food products in order to extend their shelf-live and create new attractive taste products (Dadalioglu and Evrendilek, 2004 and Schmidt et al., 2005). As a matter of fact, spices and herbs have long been used in foods not only for their flavoring and enhance food palatability, but also for their medicinal and preservative properties (Davidson el al., 1983). They are used for preventing food spoilage, deterioration and extending shelf-life of food (Nakatani, 1994 and Yusef, 2018) Because of consumer demand for less use of artificial food additives, research has led to use Safe naturally substances as alternatives

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to synthetic preservatives which can be applied in food industry (Gould, 1996). Therefore, understanding and analyzing the properties of natural antimicrobial substances are critical for food processing (Madsen and Grypa, 2000). Antimicrobial extracts are non-nutritive chemical substances derived from different parts of the plant (eg. seed, leaf, flower bud, fruit, bark or rhizome). They could be soluble in water or organic solvent and oil.

Antimicrobial activity of plant extracts or volatile oils is dependent, mainly, on their chemical composition. natural antimicrobial substances in some spices are considered as powerful tools to generate appetites, inhibit cholesterol synthesis, block estrogen, lower blood pressure and prevent blood clotting (Uhl, 2000 and Khameeneh et al., 2019). In addition, they play an important role in promoting human health by their anticancer, antioxidative, anti-inflammatory properties (Wiseman et al.,1997 and (Gashaw et al., 2020). prevent cardiovascular diseases and aging (Balentine et al., 1999).

Accordingly, the aims of this study are:

- To figure out the optimum conditions for extracting materials having the characteristics of natural antimicrobial substances from the previous spices.
- To determine the efficiency of some commercially available spices in the Egyptian local market (e.g. cinnamon, clove, fennel and thyme) and their aqueous or ethanolic extracts to be further use as antimicrobials in laboratory media against gram-negative bacteria such as Escherichia coli, gram-positive bacteria such as *Staphylococcus aureus* and pathogenic bacteria such as *Salmonella typhimurium*.

2. Materials and Methods

Materials

Spices plants

Spices and herbs illustrated in Table 1. were obtained from Medicinal and Aromatic Research Department, Agricultural Research Center (ARC), Giza, Egypt.

Table 1. The common and botanical names of the chosen spices

Common Name	Botanical Name	Family	Used part
Cinnamon	Cinnamomum verum	Louraceae	Bark
Clove	Syzygium aromaticum	Myrtaceae	Flower bud
Thyme	Thymus vulgaris	Lamiaceae	Flowering tops
Sweet Fennel	Foeniculum vulgare	Apiaceae	Fruit

Methods

Preparation of plant extracts

Water and ethanolic extracts of different plants under this study were prepared according to the method described by (Atta and Imaiznmi, 1998). Plants were normally, cleaned and ground in an electrical grinder to pass through a 0.42 mm sieve. Then, The milled samples were stirred individually for 30 min with 5 volumes of solvent in a 500 ml covered conical flask at room temperatures. After that the suspension was centrifuged at 3500 rpm for 10 min and the supernatant was filtered through a cotton cloth and the extraction was repeated twice with the solid residue. Finally, the combined extract was concentrated via vacuum evaporation and dried at 50°C in an electric oven, then weighed to determine the extraction yield and stored at -18° C until further use.

Factors affecting extracted yield

Extraction procedure was repeated at the same manner with change solvent to sample ratio (1:5, 1:10, 1:15 and 1:20 w/v) for different stirring period (1, 2, 3 and 4 hours) and using different concentrations of ethanol in water (80, 60, 40, 20 and 0%). As a result, optimum conditions for maximum extraction of antimicrobial compounds from plant samples were figured out.

Determination of some phytochemical constitutes in tested spices and herbs

Phytochemicals constitutes of different tested spices and herbs including; total soluble polyphenols; flavonoids, alkaloids and saponin were determined in their alcoholic extracts (80%).

Determination of alkaloids

Alkaloids content of different alcoholic extracts were determined by the method described by Ruiz, 1978) with some modification as follows. Two ml from each of spices and herbs ethanolic extract was placed in a conical flask, mixed with 10 ml ammonical chloroforms (3:1v/v) and 1 ml concentrated ammonium hydroxide was added. The sample was then collected and added or completed with 25 ml of chloroform (2.5 x 4). Alkaloids were extracted from the chloroform layer by shaking with 80 ml of 0.1 N sulfuric acid using separatory funnel, divided into portions and shacked to mix them thoroughly until the alkaloids removed from the solvent. The extract was basified with NH_4OH and then extracted by shaken with several successive portions with chloroform (10 ml x 2) in a separator funnel. The solvent was removed by a rotary evaporator under reduced pressure. The residue was dried in electrical oven at 60°C for two hours then weight to determine the alkaloids on dry weight basis.

Determination of Saponines

Saponines of different alcoholic extracts were performed colorimetrically according to the method described by (Hiai et al., 1976) as follows. Aliquot of 5 ml from each of spices and herbs extract was placed into 15 ml test tube. Added 0.5 ml of vanillin solution (800 mg of vanillin was dissolved in 10 ml of 99.5% ethanol) and 50 ml of sulfuric acid 72%. The mixture was warmed in a water bath at 60°C for 10 min, and then cooled in an ice-cold water. Absorbance was recorded against the blank by using the previous spectrophotometer at 544nm. Saponine content was expressed as mg/100 g dried plant extract.

Determination of total flavonoids

Total flavonoids of crude extract in different from each of spices and herbs were determined according to the method described by (Herrmann 1976; Zhishen et al., 1999; and Kim et al., 2003) as follows: Aliquot of 5 ml from each of spices and herbs extract was placed into a 10 ml volumetric flask containing 4 ml distilled water then addition, 0.3 ml 5% $NaNO_2$ and shake. After 5 min, 0.3 ml 10% aluminum chloride was added. After 6 min., 2 ml 0.1 M sodium hydroxide was added. Immediately, the mixture of the flask was diluted to a volume of 6 ml with distilled water to reach the mark of the flask and thoroughly mixed. Absorbance of the mixture (pink color) was measured at 510 nm using spectrophotometer (Spectroonic 20, Germany). Catechol was used as standard reagent. Total flavonoids content of sample was calculated using the following linear equation based on the calibration curve:

$$Y = 0.010205\ x - 0.0279 \qquad (1)$$

Where:

Y = absorbance of the sample.

X = the flavonoid content as mg/100 g.

Determination of total polyphenolic acids and free phenols

Total polyphenolic acids and free phenols compounds in the crude alcoholic extracts of different spices and herbs were determined according to the following the method of (Jidel and Singh, 1975) as follows: Aliquot of 5 ml of the extracts was added to 5 ml of distilled water in screw capped test tubes followed by addition of 0.1 ml of folin Denn's reagent. Let the mixture to stand 3 min at room temperature and then added 0.1 ml 20% sodium carbonate solution. Let the mixture to stand 30 min at room temperature blue color was developed. Shake, then the absorbance of the mixture, was measured at 650 nm. Tannic acid was used as a standard phenol. The results were expressed as mg phenol/g dry weight of plant.

Statistical analysis

Data were analyzed with the statistical analysis software, CoStat (2005). All multiple comparisons were first subjected to analysis of variance (ANOVA). Comparisons among means were made using least significant differences (L.S.D.) at $P \leq 0.01$ and 0.01 according to Steel and Torrie (1980)

3. Results and Discussion

Optimization of extraction conditions Impact of extractants on total soluble solids (TSS) yielded from different spices and herbs

Water is a universal solvent which is usually used to extract water-soluble substances. Alcohol is also could be used as an organic solvent to extract different chemicals components from the dried spices and herbs. As shown in Table 2., the yield of studied spices was varied according to the type of solvent and sort of spices and herbs. Since alcoholic solvents were more effective than water to increase the amount of extracted substances.

Data in Table 2. show, that ethanol in general, was more suitable for TSS recovery than water. Moreover, 80% ethanol showed significant increase and was found to be the most effective in TSS recovery during the extraction process than the other concentrations for clove, cinnamon and thyme. The values of TSS (recovered by 80% ethanol) were 18.2, 16.72 and 16.33 g/100 g plant from clove, cinnamon and thyme, respectively. On the opposite, using 80% ethanol as a solvent for fennel did not record any significant differences in TSS compared with water which extracted the highest TSS from the fennel. Our results are in accordance with those obtained by (Nascimento et al., 2000), who used 70% ethanol extracts on 30 plant species. These results also were agreed with these reported by (Baratta et al., 1998, Naidu 2000, Mau et al., 2001 and Cowan, 1999) whom indicated that all of the identified compounds from spices and herbs were aromatic or saturated organic compounds had an activation against microorganisms . Therefore, they most often obtain throng initial ethanol or methanol extraction. In fact, many studies avoid the use of aqueous fractionation altogether. The high amount of alcoholic yield may be related to the ability of alcohol to extract both aromatic and saturated organic compounds beside of protein and monosaccharide. On the other hand, water extract only protein, some soluble saccharides and tannins only (Cowan, 1999). As a result, type of solvent could be play an important role in the nature of the structure and amounts of the yield (Wanger et al., 1984). In general, the extent of the inhibitory effect of plant extracts is attributed to the presence of volatile oils, which have an aromatic nucleus containing polar functional group (Gould, 1996 and Pandey et al., 2021). So the extraction of such compounds depend upon their structure.

Tabe 2. Effect of ethanol percentage in the aqueous solvent on the total soluble solids (TSS) recovered from the studied spices.

Extractant		TSS (g/ 100 g spices and herbs) on dry matter			
		Clove	Cinnamon	Thyme	Fennel
Water		8.14^{c} ±0.14	2.58^{d} ±0.02	9.87^{d} ±0.1	9.17^{a} ±0.1
Ethanol (%)	20	9.51^{b} ±0.1	3.36^{d} ±0.38	10.85^{c} ±0.1	8.05^{b} ±0.1
	40	9.88^{b} ±0.12	4.90^{c} ±0.1	13.67^{b} ±0.1	7.70^{b} ±0.1
	60	9.90^{b} ±0.2	9.60^{b} ±0.1	14.07^{b} ±0.2	6.62^{c} ±0.1
	80	18.20^{a} ±0.2	16.72^{a} ±0.1	16.33^{a} ±0.2	9.00^{a} ±0.3

Means ± standard division of triplicate trials
Within column, means having the same superscript letters are not significantly different at 1% level

Impact of extraction time on TSS yield

Effect of extraction time on TSS yield was also studied using different concentration of ethanol and water. Data in Table 3. clearly showed that relationship between time and TSS recovery. Whereas, the extend the extraction time, the higher the TSS yield. It is obvious that the amount of TSS extracted from clove was (18.20 g/100 g plant) after 4 hours of extraction which represented the highest percent among those of the other studied spices. On the other hand, fennel displayed the lowest amounts (12.31 g/100g plant) of TSS at the same time of

extraction. The time of extraction may determine the kind and nature of the antimicrobial compounds as well as their structure. These results are in agreement with the data obtained by Wanger et al., 1984) and Martini et al., 1996) those reported that flavonoids lacking hydroxyl groups on their β-rings are more active against microorganisms than are those with the -OH groups, and microbial target is the membrane. Lipophilic compounds would be more disruptive of this structure. However, other author has also found opposite effect, the more hydroxylation, the greater the antimicrobial activity (Cowan 1999 and Prastiyanto et al., 2020).

Table 3. Effect of extraction time on TSS recovered from studied spices at room temperature.

Time of extraction (Hour)	TSS (g/ 100 g plant)			
	Clove	Cinnamon	Thyme	Fennel
1	18.20^{b} ±0.2	16.72^{c} ±0.1	16.33^{b} ±0.2	9.00^{c} ±0.3
2	18.20^{b} ±0.1	17.29^{b} ±0.2	16.52^{b} ±0.3	10.98^{b} ±0.02
3	19.76^{a} ±0.2	18.31^{a} ±0.2	19.54^{a} ±0.06	11.89^{a} ±0.1
4	20.10^{a} ±0.4	18.54^{a} ±0.06	20.05^{a} ±0.1	12.31^{a} ±0.1

Means ± standard division of triplicate trials
Within column, means having the same superscript letters are not significantly different at 1% level.

Effect of solvent ratio on TSS yield impact from spices and herbs

Influence of plant : solvent (w/v) ratio on TSS recovered from the studied spices and herbs after 4 hours extraction at room temperature was also investigated. The data in Table 4. reveal that as the plant to solvent ratio increased, TSS yield was augment. Therefore, the highest yield from clove and cinnamon was achieved at 1:10 ratio. On the other hand, thyme and fennel at ratio of 1:20 achieved the maximum recovery of TSS. It is of special important to note that thyme and fennel : ethanol solvent at 1:20 ratio extracted 30.39 g and 15.28 TSS/100 g from fennel. Plant extracts are complex mixtures of different compounds.

Table 4. Effect of plant: solvent ratio on TSS recovered from the studied spices and herbs at room temperature for 4 hours.

plant : ethanol ratio	TSS (g/ 100 g plant)			
	Clove	Cinnamon	Thyme	Fennel
1:5	21.60^{a} ±0.4	18.54^{a} ±0.06	20.05^{d} ±0.1	12.31^{d} ±0.1
1:10	21.63^{a} ±0.07	19.20^{a} ±0.2	25.68^{c} ±0.2	13.55^{c} ±0.3
1:15	21.60^{a} ± 0.4	18.21^{a} ±0.3	27.41^{b} ±0.2	14.73^{b} ±0.3
1:20	8.89^{b} ±0.09	8.11^{c} ±0.1	30.39^{a} ±0.2	15.28^{a} ±0.2

Means ± standard division of triplicate trials
Within column, means having the same superscript letters are not significantly different at 1% level

Determination of some phytochemicals in the tested spices and herbs

The chemical composition of secondary metabolites phytochemicals namely phenolic compounds, saponine, flavonoids and alkaloids which expected to have antibacterial activity, were determined in clove, cinnamon and thyme plants. Data of Table 5. show that no significant difference in phenolic contents between clove and cinnamon. While thyme contained the lowest amounts of phenolic compounds. Concerning saponin contents in the tested plants, the data show significant differences among the three plants. Whereas the highest content (9.41 mg/g dry wt.) was found in clove while, the lowest

(3.14 mg/g dry wt.) was detected in thyme. The same trend was observed with flavonoids and alkaloids, where significant differences were found among the chosen plants. However, such chemical composition of the tested plants may explain their antibacterial effect on the tested microorganisms (Manandhar et al., 2019 and Elisha et al., 2017). The mechanism of action for phenolic and flavonoids compounds on microbe is due to their binding with cell wall and inactivate enzymes. While, the alkaloids were interpolate into the cell wall and /or bind with DNA (Houghton et al., 1994 and Freiburghaus et al., 1996).

Table 5. Phytochemical contents of the ethanolic extracts recovered from the tested spices.

Plant	Phytochemical contents (mg/g on dry weight)			
	Saponines	Flavonoids	Alkaloids	Phenolic acid
Clove	9.410^{a} ±0.01	16.99^{a} ±0.09	0.074^{b} ±0.001	0.199^{a} ±0.001
Cinnamon	4.955^{b} ±0.045	10.55^{b} ±0.45	0.058^{b} ±0.001	0.190^{a} ± 0.001
Thyme	3.136^{c} ±0.004	2.60^{c} ±0.1	2.630^{a} ±0.02	0.177^{b} ±0.002

Means ± standard division of triplicate trials
Within column, means having the same superscript letters are not significantly different at 1% level

Referring to Table 5. the concentration of phenolic compounds saponine and flavonoids of cloves ethanolic extract were higher than those found in cinnamon ones. While the lowest values were recorded by thyme alcoholic extract. On the other hand, thyme contained the significant highest amounts (2.63) of alkaloids among the other studied plants. These results are in agreement with those found by (Naidu 2000 and Özdal et al., 2019). who reported that major antibacterial components of clove was Eugenol. They were cinnamic aldehyde and eugenol in cinnamon and thymol and carvacrol in thyme. Cinnamon is rich in essential oils and tannins which inhibit microbial growth (Bullerman et al., 1977). Meanwhile, thyme essential oils, which contain the terpenes, carvacol and thymol, has inhibitory effect against the growth of many pathogenic bacteria e.g., E. coli and S. typhimurium (Davidson, 1997 and Sikkema et al., 1995). Added to that, thyme extracts disintegrate and disrupt the cytoplasmic membrane permeability and reduced the intercellular ATP (Ultee et al., 1999 and Mossebo et al., 2020).

4. Conclusion

Ethanol in general, was more suitable for TSS recovery than water. Moreover, 80% was found to be more effective in TSS recovery during the extraction process than the other concentrations for clove, cinnamon and thyme. But, using 80% ethanol as a solvent for fennel did not record any significant differences in TSS compared with water extract. There was directly relationship between time and TSS recovery. Where, clove extract surpassed the other spices extracts at the all tested time intervals. Clove was the best after 4h. The decreasing in plant : ethanol ratio (down to 1:10) the increasing of TSS recovered from both clove and cinnamon, then decreased sharply at 1:20 ratio. Thyme and Fennel at ratio of 1:20 achieved the maximum recovery from TSS. Generally, the high contents of phenols, saponines and flavonoids were determined in clove plant followed by cinnamon, while thyme came at the end. However the low content of alkaloids was found in cinnamon plant followed by clove then thyme.

References

Amit, K.S., Mezbah Uddin, M. D., Rahman R., Rezwanul Islam S. M. & Khan S. M. (2017). A review on mechanisms and commercial aspects of food preservation and processing. Agriculture and food secure 6(51), 1-22.

Atta, M. B. & lmaizumi, K. (1998). Antioxidant activity of nigella (*Nigella stativa* L.) seeds extracts. J. Jpn. Oil Chem. Soe, 47:475- 480.

Balentine, D. A., Albano M. C., & Nair M. G. (1999). Role of medicinal plants, herbs, and spices in protecting human health. Nutr. Rev., 57, 41-45.

Baralta, M. T., Dorman, H. P., & Deans S. G. (1998). Chemical composition, antimicrobial and antioxidant activity of lounel sage, rosemary, oregano and coriander essential oils. J. Essent. Oil Res., 10, 618-627.

Branter, A., & Grevn, E. (1999). Antimicrobial activity of plant extracts used externally in traditional medicine, J. Ethnopharmacol, 44, 35-40.

Bullerman, C.U., Lieu, .FY., & Seier, S. (1977). Inhibition of growth and aflatoxin production by cinnamon and cloves oils, cinnamic aldehyde and eugenal, J. Food Sci., 42, 1107-1109.

CoStat, (2005). CoHort Software, version 6.311. 798, Lighthouse Ave. PMB 320 Monterey, CA, 93940, USA.

Cowan, M. M.(1999). Plant products as Antimicrobial Agents. *Clin. Microbiol. Rev*. 12, 564-582.

Dadalioglu, I., & Evrendilek, G. (2004). Chemical compositions and antibacterial effects of essential oils of Turkish oregano (*Origanum minutiflorum*), bay laurel (*Laurus nobilis*), Spanish lavender (*Lavandula stoechas* L.), and fennel (*Foeniculum vulgare*) on common foodborne pathogens. J. Agric Food Chem 52, 8255–8260

Davidson, M. P. (1997). Chemical preventives as natural antimicrobial compounds. In Doyle LR, Beuchat and Montrille TJ (eds), "Food microbiology Fundamentals and Frontiers ASM press, Washington DC. Pp. 520-555.

Davidson, P. M., Post, O. L. S., Branen, A. L., & Mccurdy, A. R. (1983). Naturally occurring and miscellaneous food antimicrobials. In: *Branen AL and Davison PM (eds.)* Antimicrobials in Food, 371-419, Marcel Dekker, New York.

Elisha, I. L., Botha, F. S., & McGaw, L. J. (2017). The Antibacterial Activity of Extracts Of Nine Plant Species With Good Activity Against Escherichia Coli Against Five Other Bacteria and Cytotoxicity of Extracts. BMC Complem Altern M 17:133.

Freiburghaus, F., Kaminsky, R., Nkunya, M. & Brun, R. (1996). Evaluation of African medicinal plants for their in vitro trypanocidal activity. J. Ethopharmacol. 55:1-11

Gashaw, G., Fassil, A., & Redi, F. (2020). Evaluation of the antibacterial activity of Pleurotus Spp. Cultivated on different Agricultural wastes in Chiro, Ethiopia. Int. J Microbiology, V(2020), P:1-9.

Gebreyohannes G., Nyerere A., Bii C., & Sbhatu B.D. (2019). Investigation of Antioxidant and Antimicrobial Activities of Different Extracts of Auricularia and Termitomyces Species of Mushrooms. Sci World 1-11

Gomez, K. N., & Gomez, A. A. (1984). Statistical Procedures for Agricultural Research. John Wiley and Sons, New York, 2nd ed.

Gould, G. W. (1996). Industry perspectives on the use of natural antimicrobials and inhibitors for food applications. J. Food Prot. (suppl.), 82–86

Herrmann, K. (1976). Flavoniods and flavones in food plants. A review, J. Food Technol. 11, 433 -448.

Hiai, S., Oura, H., & Nakajima, T. (1976). Color reaction of some sapogenins and saponines with vanillin and sulluric acid. Planta Medical 29, 116-122.

Jidel, K. K., & Singh R. N. (1975). Phenolic content in male and female carica papaya a possible physiological marker for sex identification of vegetative seed lings. Physical. Plant. 33,104-107.

Khameeneh B., Iranshahy M., Soheili V., & Bazzaz F.S.B. (2019). Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob Resist Infect Control . 8(1) P:1-28.

Kim, D. O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plum. Food Chem. 81:321-326.

Madsen, M. G., & Grypa, R.D. (2000) Spices, flavor systems, the electronic nose, Food Technol. 54, 44-46.

Manandhar, S., Luitel, S., & Dahal, R. K. (2019). In vitro antimicrobial activity of some medicinal plants against human pathogenic bacteria. J. Trop Med 5, 1-5.

Martini, H., Weidenborner, N., Adams, S. Kunz, B. (1996). Eugenal and caruacrol: the main fungicidal compounds in clove and savory. Italian J Food Sci., 8,03-04.

Mau, J., Chen C., Hsieh P., Mau J. L., Chen C. P. & Hsieh P.C. (2001). Antimicrobial effect of extracts from Chinese chive cinnamon and corni fructus. J. Agric. Food Chem. 49,183-188.

Mossebo, D. C., Metsebing, B. P., & Oba, R. (2020). Comparative evaluation of antifungal and antibacterial activities of crude extracts of Pleurotus sajor-caju, Pleurotus tuber-regium and Lentinus squarrosulus (Basidiomycota, Pleurotaceae, Lentinaceae) from Cameroon. EJBIO.1(5):1-7.

Naidu, A. S. (2000). Natural food Antimicrobial system. C R C press Boca Raton London New York Washington D. C USA chapter 3pp 265-385.

Nakatani, N. (1994). Antioxidant and antimicrobial constituents of herbs and spices. In: Charalambous G (ed.) Spices, Herbs and Edible Fungi, pp.251-271, Elsevier, New York.

Nascimento, G. G. F., Locatelli, J., Freitas, P. C., & Silva G. L. (2000). Antimicrobial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. J. microbial., 31, 247-256.

Özdal, M., Gülmez, Ö., Özdal, G. (2019). Antibacterial and Antioxidant Activity of Mycelial Extracts of Different Pleurotus Species. Food Health 5, 12–18.

Pandey, A. T., Pandey, I., kinase, A., Verma, A., & Garcia-Canibbano, B. (2021). Validating anti-Infective activity of Pleurotus Opuntiae via standardization of its bioactive mycoconstituents through multimodal. Biochemical Approach. 11(484):1-18.

Prakash, K. D., & Sayali, G. (2017). Current Quality Control Methods for standardization of Herbal Drugs. Int. J. Pharm 5, 82–95.

Prastiyanto, M.E., Rukmana, R. M., Saraswati, D. K., Darmawatis, Maharani E. T. W, & Tursinawati, Y. (2020). Anticancer Potential of Methanolic Extract From Pleurotus Species On Raji Cells and Antibacterial Activity Against Methicillin-Resistant Staphylococcus aureus. Biodiversitas, 21(12), 5644-5649

Schmidt, E., Jirovetz L., & Buchbauer G. (2005). Antimicrobial testing and gas chromatographic analysis of aroma chemicals. J. Essential Oil Bearing Plants 8, 99–106.

Sikkema, J. J., De Bont A. M., & Poolman B. (1995) Mechanism of membrane toxicity of hydrocarbons. Microbiol, Rev. 59, 201-222.

Steel, R. G., & Torrie J.H. (1980). Principles and procedures of statistics. 2nd ed. (pp-120) MC Graw- Hill, New York, USA.

Uhl, S., (2000). Spices: Tools for Alternative or Complementary Medicine. *Food Technol*. 54: 61-62, 64, 65.

Ultee, A., Bennik, M. & Moezelaar, R. (2002). The phenolic hydroxyl group of carvacrol is essential for action against the foodborne pathogen Bacillus cereus. Appl. Environ. Microbiol 68:1561–1568.

Wanger, H., Blandt, S. & Zgainski, E. M. P. (1984) Plant Drug Analysis. Spring-Veriay, New York P 320-331.

Wiseman, S. A., Balenttine, D. A. & Frei, B. (1997). Antioxidants in tea. Cri. Rev. Food Sci. Nutr. 37, 705-718.

Yusef, M. (2018). Chapter 12 "Natural Antimicrobial Agents for Food Bio preservation "in "Food Packaging and Preservatives Hand Book of Food Engineering". YMD college, Maharshi Dayanand, Nuh, Haryana, India, 409-438.

Zhishen, J., Munching, T. & Jianming, W. (1999). The determination of flavonoids contents in mulberry and their scavenging effects on superoxide radical. Food Chem., 64:555-559.