World Journal of Advanced Research and Reviews, 2020, 05(01), 058–066

World Journal of Advanced Research and Reviews

e-ISSN: 2581-9615, Cross Ref DOI: 10.30574/wjarr

Journal homepage: https://www.wjarr.com

(RESEARCH ARTICLE)

Physicochemical properties and inhibitory effects of essential oils from selected local Spices

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Publication history: Received on 22 December 2019; revised on 21 January 2020; accepted on 23 January 2020

Article DOI: https://doi.org/10.30574/wjarr.2020.5.1.0110

Abstract

Spices are rich in essential oils and are known to possess antiviral, antibacterial, antifungal and insecticidal properties. This study evaluated the inhibitory effect and physicochemical properties of essential oil from some selected spices. Cinnamon, ginger and garlic were pulverized and extracted using the Soxhlet method with n-Hexane. The extracted oils were subjected to physicochemical and microbial analysis. Results showed that ginger gave the highest oil yield (7.01%) in comparison with cinnamon and garlic: 2.75 and 1.33 % oil yields at respective 10.62, 14.29and 11.16 % moisture contents. Iodine value, acid value and free fatty acid value was significantly ($p<0.05$) higher in cinnamon oil (506.15, 20.66 mgKOH/g and 10.33 mgKOH/g respectively) compared to the respective 348.65, 15.41 mgKOH/g and 7.71 mgKOH/g for ginger and 128.15, 17.90 mgKOH/g and 8.95 mgKOH/g for garlic. Saponification value was significantly ($p<0.05$) higher in ginger (226.15mgKOH/g) than in cinnamon (214.75 mgKOH/g) and garlic (198.54 mgKOH/g). A significantly high ($p<0.05$) peroxide value was observed in garlic with 247.12 milli eq/Kg, and 234.15 and 247.12 milli eq/Kg for cinnamon and ginger respectively. Cinnamon oil was observed to be the most potent against the tested microorganisms, showing maximum inhibition zones of 0.9 cm for *E. coli*, 1.1 cm for *P. aeruginosa* and 2.4 cm for each of *K. pneumonia*, *Serratia spp.* and *S. aureus.* Result from the study revealed that the extracted oils are not fit for consumption, highly susceptible to rancidity and oxidation but are of good potential use in paint and soap making.

Keywords: Cinnamon; Ginger; Garlic; Essential oil

1. Introduction

Essential oils (EOs) are plant-derived volatiles with a hydrophobic character. They have been defined as intricate mixtures of volatile, odoriferous, lipophilic and liquid substances [1; 2]. Essential oils, being products of secondary plants' metabolism play important roles in plant protection such as antiviral, antibacterial, antifungal, insecticidal properties, also against herbivore attack. Majority of them are used as seasonings and medicines [2; 3].According to [4],of about 3,000 essentials oils which are currently known, approximately 300 of them are commercially important in the food, sanitation, pharmaceutical, agronomic, cosmetics, and perfume industries.

Various additives are used to extend the storage period of foods and to prevent the growth of microorganisms [5]. In spite of that, there is an immerse interest in the use of alternatives, mainly natural products, such as plants and spices [6; 7]. Spices are rich in essential oils and extracts, with established antimicrobial activity. Due to the recognition of the antimicrobial properties for the past years [8], materials can be used to suppressor delay the emergence of microorganisms. Most herbs and spices exhibit antimicrobial activity due to the fractions of their essential oils. Several EOs from medicinal and aromatic plants have been known since ancient years to possess biological activity, ,, antibacterial and antioxidant properties [9; 10; 11].Essential oils can be obtained through fermentation, expression, effleurage or extraction but according to [12] the most generally used method for mass production is the steam method.

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Basically, the antimicrobial activity of plant oils and their extracts have become the basis of various applications including raw and processed food preservation, pharmaceuticals, medicine and natural therapies [8]

The aim and objective of this study was to determine the inhibitory effect of essential oils gotten from three (3) different spices against some food spoilage microorganisms and to ascertain the physicochemical properties of the essential oils with a hope to further the study for food preservation and storage.

2. Material and methods

All reagents used for this research work were of analytical grade unless otherwise stated. This research work was carried out at the Chemistry/Biochemistry and Microbiology laboratories of the Nigerian Stored Products Research Institute, Ilorin. Kwara State.

2.1. Sample collection and preparation

Cinnamon, ginger and garlic were purchased from Yoruba Road market in Ilorin. Ginger and garlic samples were peeled, washed and sliced to small pieces, Cinnamon bark was cleaned and broken to pieces, the samples were then oven dried at 50oC to a constant weight. The samples were pulverised with the aid of hammer mill. The milled samples were placed in neat and well labeled air tight plastic bottles and kept for further use.

2.2. Determination of moisture content

The moisture content determination was carried out adopting the [13] methods. Weighed portions (5g) of the milled samples were dried in an oven to a constant weight at 105^0C for 5 hours.

2.3. Oil extraction and oil yield determination (%)

The oil content of each sample was determined separately by complete extraction using the Soxhlet extractor (Konte, USA).50g of milled samples were placed into a porous thimble in a soxhlet extractor, using n-hexane (150mL) as the extracting solvent for 6 hours. The oil was obtained after the solvent was removed under reduced temperature and pressure and refluxing at 70^oC to remove the excess solvent from the extracted oils [14]. The oils were placed in bijou bottles and kept for further use.

The % oil yield was determined by placing the extracted oils over water bath for 30min at 70^oC to ensure complete evaporation of solvent and weight of the oil was recorded and expressed as oil content as described by [13]. The oil yield (%) was calculated as thus:

$$Oil\ yield\ (\%) = \frac{weight\ of\ oil}{weight\ of\ sample} X100$$

2.4. Physicochemical analyses

The physicochemical analyses of the oils were determined using standard methods as reported in each of the parameters. The parameters analysed were refractive index, saponification value, acid value, free fatty acid, peroxide value and iodine value and the methods of analysis used are as follows:

2.5. Saponification value

As described by [14], 2g of each oil sample was added to a flask with 30 mL of ethanolic KOH and was then attached to a reflux condenser for 30minutes to ensure that the sample was fully dissolved. After sample was cooled, 1ml of phenolphthalein was added and titrated against 0.5 M HCl until a pink colour appeared which indicated the end point. A blank was also carried out which contains all reagents but no oil and was titrated against 0.5M HCl. The saponification value was calculated as thus:

$$Saponification\ (mgKOH/g) = \frac{56.1\ N\ (Vo - V1)}{M}$$

Where V_0 = Volume of the solution used for blank test

V_1 = Volume of the solution used for sample

N = Actual Normality of the HCl used

M = Mass of the sample.

2.6. Refractive index

The refractive indices, η/D^{30} (RI), of the crude oil samples were measured using an Abbe refractometer at 30±0.1°C. 20°C.

2.7. Iodine value (IV)

The Iodine Value of the oils were determined using a mathematical relationship between refractive index and iodine value has been described by [15] and reported by [16] as η/D^{30}Refractive index (RI) = 1.45765+0.0001164 IV (Iodine value). The relationship was adjusted and used to calculate the iodine value of oils since the refractive indices are is known as follows:

$$\text{Iodine Value } (gI_2/100g) = \frac{\text{Refractive Index} - 1.45765}{0.0001164}$$

2.8. Acid value/free fatty acid

25ml of neutral ethanol was heated to boiling and added to 1g of each oil extract to dissolve in a conical flask. The heating was stopped and the solution was titrated with 0.1MNaOH solution using phenolphthalein as indicator [16].

$$Acid\ Value\ (mgKOH/g) = \frac{Titre\ X\ 5.61}{weight\ of\ oil\ used}$$

The free fatty acid of the oil can be determined from the expression of:

$$\text{Free Fatty Acid (\% as Oleic acid)} = \frac{Acid\ Value}{1.99}$$

2.9. Peroxide value

The peroxide values of the extracted oils were determined using the standard method described by [17]. Exactly 1.0g of KI and 20ml of solvent mixture (glacialacetic acid: chloroform, 2:1 v/v) were added to 1.0g of the oil sample and the mixture was boiled for one minute. The hot solution was poured into a flask containing 20ml of 5% KIO3solution. Few drops of starch solution were added to the mixture and the latter was titrated with 0.025M $Na_2S_2O_3$solution. A blank was also carried out and titrated with 0.025M $Na_2S_2O_3$.

2.10. Microorganisms used and standardization of the inoculums

The experiment was undertaken using a modification of [18], basically the agar well diffusion assay in a duplicate setup.

2.11. Extract description

Three plant extracts presented as Garlic, Ginger and Cinnamon were evaluated for antimicrobial efficacy against five clinical/food bacterial isolates.

2.12. Culture media used

2.12.1. Nutrient Agar

28g of the commercial medium (Lab M Limited, Lancashire) was dissolved in 1000mL of distilled water, homogenized and sterilized at 121°C for 15mins in the autoclave. Molten medium was allowed to cool before pouring.

2.12.2. Mueller Hinton Broth

21g of the product (HiMedia, India) was weighed into 1000ml distilled water; the mixture was homogenized on a laboratory hot plate with repeated swirling before sterilization in the autoclave at 121°C for 15mins.

2.12.3. Mueller Hinton Agar

28g of the commercial medium (Oxoid, UK) was dissolved in 1000ml distilled water; with frequent agitation, the solution was homogenized on the magnetic hot plate and autoclaved at 121°C for 15mins. Upon cooling 40 - 45°C, the molten medium was dispensed into sterile petridishes on a uniform laboratory bench to achieve uniform depths in the agar plates.

2.13. Test microorganisms and inoculums preparation

The identified bacterial species: Escherichia coli, *Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia sp*. and *Staphylococcus aureus* were obtained from the University of Ilorin Teaching Hospital on nutrient agar slants. From the overnight bacteria cultures, 2 – 4 colonies respectively was discretely suspended in 15ml Mueller Hinton Broth and incubated at 37°C overnight. Each test tube containing 5ml bacteria suspension had turbidity adjusted to 0.5 g MacFarland standard of 1.5 x 108cfu/ml.

2.14. Sensitivity test

Solidified Mueller Hinton Agar plates were welled with a 0.8cm sterile stainless-steel cork borer; the bored agar plates were seeded with 3ml respective bacteria inoculums, swabbed over the entire agar surface using sterile swab sticks.

Using a micropipette, 500µL of each extract was dispensed into the wells occupying the centre of each agar plates; covered with the Petri dish lid, plates were incubated in the incubator at 37°C for 18 – 24 hours. Control plates lack the incorporated extracts.

Observed zones of inhibition were accurately measured using a laboratory meter rule and recorded appropriately.

2.15. Statistical analysis

All analysis was carried out in three replicates, except stated otherwise. Data was subjected to analysis of variance (ANOVA) and tested for significance difference among spices by New Duncan's Multiple Range F-Test (DMRT) at ($p<0.05$) using SPSS software package version 20.0.0 (IBM Statistics).

3. Results and discussion

3.1. Physicochemical properties

The physicochemical properties of oils extracted from the selected spices are presented in Table 1. As shown below, cinnamon, ginger and garlic were extracted at 14.29%, 10.62% and 11.16% moisture contents with the resulting 2.75%, 7.01% and 1.33% yields respectively. In comparison with some conventional oil seed crops like soybean, mustard and cotton with yields in the range of 17.0 – 21.0%, 24.0-40.0% and 15.0 – 24.0% respectively, [19] yields recorded in this study was found to be relatively low. This has revealed that the selected spices cannot be regarded as oil crops.

The refractive index is a physical parameter that reveals the level of purity of oils and also explains the degree of the deflection that occurs when a beam of light passes from one transparent medium to the other [20]. The results of the refractive index of the essential oils measured were 1.52, 1.50 and 1.47 for cinnamon, ginger and garlic respectively. The value gotten for the essential oils is evidence that the samples might be of long carbon chain with high degree of unsaturation. It's also an indication that the oils are pure in order of decreasing values.

The iodine value of oil gives an indication of the degree of unsaturation of the oil. The higher the iodine values of oils, the higher the degree of unsaturation (Carbon to carbon double bonds) of the oil, which is also a good advantage in the production of soap [21]. The greater the iodine value, the higher the susceptibility of the oil to oxidation [16]. The Iodine values of the essential oils were found to be 506.15, 348.65 and 128.15 for cinnamon, ginger and garlic respectively. In this context, cinnamon oil has the highest iodine value, hence the most unsaturated of the oils, followed by ginger while garlic has the lowest iodine value and least susceptible to oxidation.

The acid values for cinnamon, ginger and garlic oils were found to be 20.66, 15.41 and 17.90 mgKOH/g respectively. These values are relatively higher than that of olive oil 17mgKOH/g as reported by [22]. Acid value indicates the amount of free fatty acids present in oils and the higher the acid value, the lower the storability and the higher the risk of the oil undergoing rancidity. Essential oils contain several volatile aroma compounds, mostly as free fatty acids. [14]. The free fatty acids present in the extracted oils as indicated below were found to be high, with cinnamon being significantly

($p<0.05$) higher than garlic which was in turn significantly ($p<0.05$) higher than ginger. The high values recorded for acid value suggests the oils not useful for skin care products [23]. Also, the free fatty acid values were found to be higher than 2.33% recorded by [16] for groundnut oil and much lower than 32.95% for corn oil. For oils to be considered edible, it should fall within the allowable limit of 1.0-3.0% for free fatty acid [24]. The free fatty acid of the oils under study suggests the oils are not suitable for consumption, highly susceptible to rancidity and have a low storability.

Table 1 Physicochemical Properties of cinnamon, ginger and garlic oils

Selected spices	Cinnamon	Ginger	Garlic
Moisture Content (%)	14.29±0.17c	10.62±0.12a	11.16±0.12b
Oil yield (% w/w)	2.75±0.08b	7.01±0.06c	1.33±0.05a
Refractive index	1.52±0.00c	1.50±0.00b	1.47±0.00a
Iodine Value	506.15±1.14c	348.65±1.52b	128.15±2.07a
Acid Value (mgKOH/g)	20.66±0.14c	15.41±0.07a	17.90±0.06b
Free Fatty Acid (mgKOH/g)	10.33±0.07c	7.71±0.03a	8.95±0.03b
Saponification Value (mgKOH/g)	214.75±0.09b	226.15±0.13c	198.54±0.10a
Peroxide Value (milli equ/Kg)	234.15±0.05b	148.60±0.17a	247.12±0.06c

Result shows mean ± standard error of triplicate readings (n=3). Means with unshared superscript in the same row are significantly ($p<0.05$) different

The saponification value measures the amount (mg) of KOH required to saponify 1g of oil. It measures the average molecular weight of all the fatty acids present in the oil. It takes advantage of hydrolyzing triglycerides of fatty acids with alkali to produce glycerol and alkali salts of fatty acids, which is of a high significant in soap making [25].The saponification values as seen in Table 1, revealed that the values ranged from 198.54–226.15 mgKOH/g with garlic and ginger having the lowest and highest value respectively. These values are relatively higher than that of beeswax (93 mgKOH/g), which is commonly used for soap making [26]. This justifies that the oils used in this study would be of good use in the production of soap.

The peroxide values of cinnamon, ginger and garlic oils are as shown in Table 1. It was discovered that the peroxide values are relatively high with a significant difference between the samples. The high values contribute to increase in rancidity rate [27]. This fact suggests that the oils understudy are highly susceptible to rancidity at room temperature.

3.2. Antimicrobial properties

The antibacterial efficacy of the respective extracts (Cinnamon, Garlic and Ginger) was tested against five bacteria species: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia sp.* and *Staphylococcus aureus* (Table 2 below). Generally, the zones of inhibition diameter ranged 0.4 – 2.5cm as revealed in Table 3 below. Qualitatively, the Cinnamon extract had the highest percentage inhibition (51.9%) against the five test bacteria, followed by Garlic (25.9%) and least value in Ginger (22.2%). The clarity of inhibition zones obtained indicates respective potency at minimal concentrations, as affirmed from cinnamon extract against *Pseudomonas aeruginosa* which was resistant to garlic and ginger extracts and the susceptibility of *Escherichia coli*, *Klebsiella pneumoniae, Serratia sp*. and *Staphylococcus aureus* to the assayed extracts.

The results obtained for antibacterial effect of this study utilizing n-Hexane as the extractant, presents higher efficacy against Pseudomonas aeruginosa than ethanolic extract of garlic as reported by [28]. The resistance of Escherichia coli to ethanolic extract of garlic as presented by the authors was subjected in the bacteria's susceptibility to n-Hexane extract in this study. The resistance of *Pseudomonas aeruginosa* was also reported by [29] to aqueous extracts of garlic and ginger; this study presents similar resistance to the n-Hexane extracts of garlic and ginger but susceptibility to cinnamon extract, although relatively lower than the garlic-ginger ethanolic extract as reported by the authors. Thus, in the qualitative yield of an extractant for antibacterial potency, n-Hexane is presented. From the results obtained, the Cinnamon extract showed the highest antibacterial potency against the test bacterial species.

Table 2 Qualitative assessment of extracts' antibacterial efficacy (based on the clarity of zones of inhibition)

Microorganisms	Extracts		
	Ginger	Garlic	Cinnamon
Escheriahia coli	+	++	+++
Klebsiella pneumoniae	+	++	+++
Pseudomonas aeruginosa	-	-	++
Serratia sp.	+	++	+++
Staphlyococcus aureus	+++	+	+++

(+++): high extract's antimicrobial potency. (++): Moderate extract's antimicrobial potency. **(+):**low susceptibility of test organisms to respective extract. **(-):** no inhibition of test microorganism/resistance to tested extract.

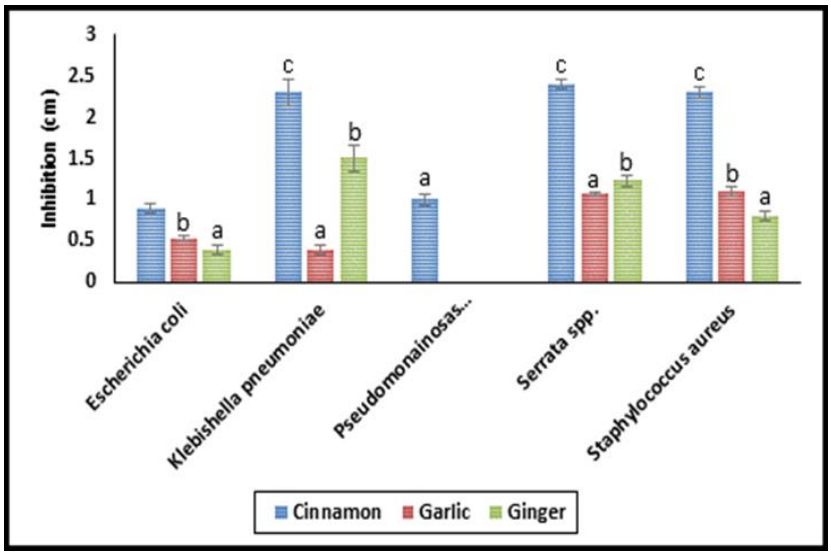

Figure 1 Effect of essential oils from cinnamon, garlic and ginger on specific microorganism

Figure 2 Zone of inhibition of Ci, Gi and Ga on *Escheriahia coli*

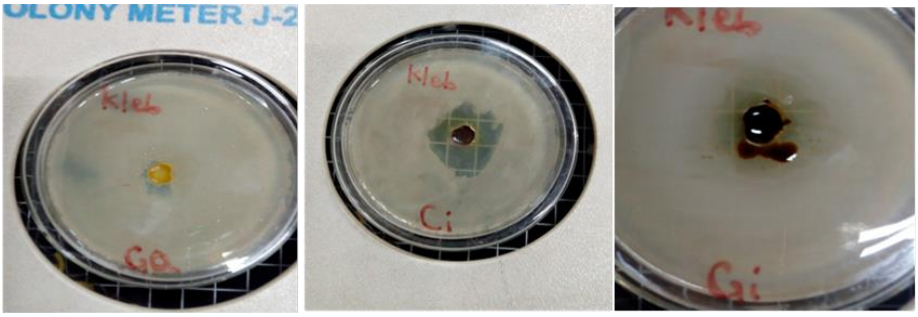

Figure 3 Zone of inhibition of Ci, Gi and Ga on *Klebsiella pneumonia*

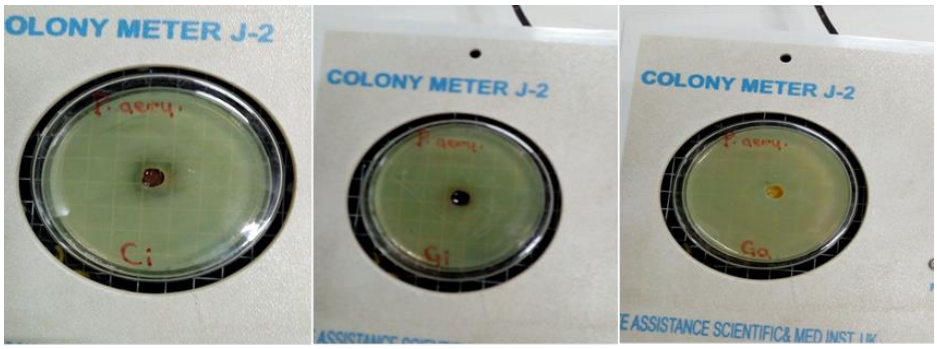

Figure 4 Zone of inhibition of Ci, Gi and Ga on *Pseudomonas aeruginosa*

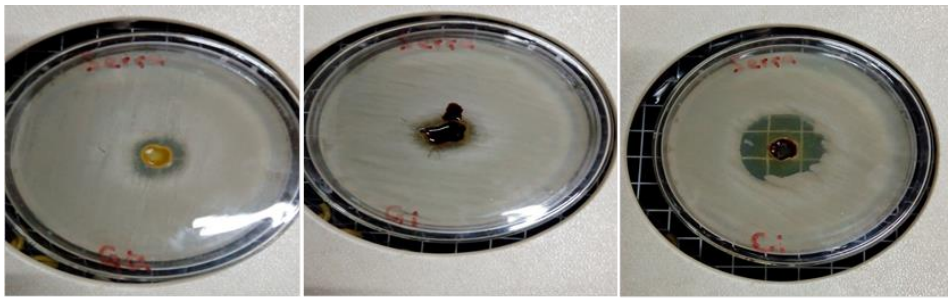

Figure 5 Zone of inhibition of Ci, Gi and Ga on Serratia sp**.**

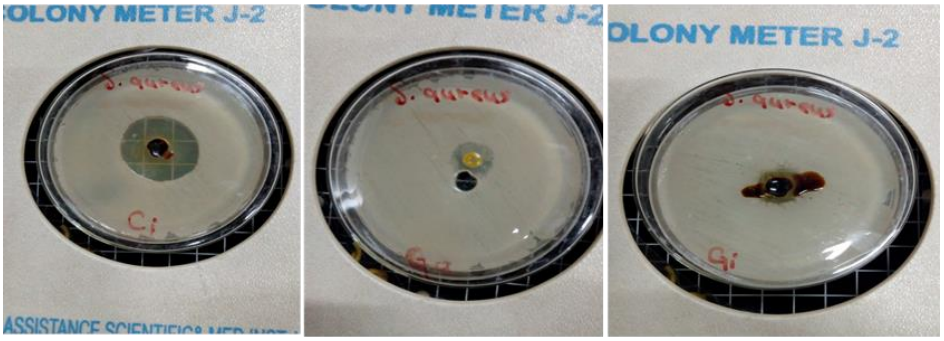

Figure 6 Zone of inhibition of Ci, Gi and Ga on *Staphlyococcus aureus*
NOTE:Ci- Cinnamon; Ga-Garlic; Gi-Ginger

4. Conclusion

The result of this study has shown that the selected essential oils are not suitable for consumption due to their high acid value and free fatty acid values but of good use in the paint, soap making and pharmaceutical companies/industries with cinnamon oil being the most important due to its highest degree of unsaturation compared to others. It can also be concluded that the selected essential oils have inhibitory effects on microorganism with cinnamon oil being the most potent amongst them, being active against all bacteria tested against it.

Compliance with ethical standards

Acknowledgments

Authors wish to acknowledge the Management of Nigerian Stored Products Research Institute, Ilorin, Nigeria, for the use of laboratory equipment during the course of this study.

Disclosure of conflict of interest

Authors have declared that no competing of interests exists.

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How to cite this article

Onyegbula AF, Lawal IO, Fashanu TA, Salawu RA, Adebayo OB and Ayanda IS. (2020). Physicochemical properties and inhibitory effects of essential Oils from selected local Spices. World Journal of Advanced Research and Reviews, 5(1), 58-66.