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Phenolic Contents, Comparative Antioxidant Studies and Anti-Tubercular Activities of Commonly Used Spices in Abuja, Nigeria (Antioxidants and Antitubercular Activities of Nigerian Spices)

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SPICES are reported to be helpful against diseases of oxidative stress such as cardiovascular diseases and exhibit antimicrobial properties. The potential for their use as adjuvants in tuberculosis cases have not been explored. In this study, the *in vitro* antioxidant and anti-tuberculosis activities of nine Nigerian spices (*Syzigium aromaticum*, *Thonnigii sanguinea*, *Piper nigrum*, *Zanthoxylum zanthoxyloides, Zingiber officinale, Cyperus articulatus, Xylopia aethiopica, Lepidum sativum,* and *Nigella Sativa*) along with their phenolic contents, including total phenolics, total flavonoid and total tannin contents were evaluated. Antioxidant activities were determined by DPPH scavenging and nitric oxide inhibitory assays. The anti-tuberculosis activity against *Mycobacterium bovis* and *Mycobacterium smegmatis* was evaluated using the broth micro-dilution method to determine the minimum inhibitory concentration (MIC). The ranges for the total phenolics, total flavonoids and total tannins contents were 7.3-193.9 mgGAE/g, 0.0-12.5 mgQE/g, and 0.0-44.4 mgTAE/g, respectively, in all spices. The extracts presented strong DPPH scavenging and nitric oxide inhibitory activities with IC_{50} values ranging from 4.2-1098 µg/mL and 0.0025-28.65 µg/mL, respectively. *S. aromaticum* and *T. sanguinea* showed significant DPPH scavenging activities with $IC_{50}$of 4.2 and 7.7 µg/mL, respectively, which were comparable to that of ascorbic acid (3.6µg/mL)($p \leq 0.05$). *Z. zanthoxyloides* and *S. aromaticum* also exhibited significant nitric oxide inhibitory activities ($p \leq 0.05$) with $IC_{50}$of 0.0025 and 0.0051 µg/mL, respectively, which were comparable to the gallic acid value of 0.00088 µg/mL. A weak anti-tubercular activity was however observed with MIC values ranging from 25000-781 µg/mL and 12500-781 µg/mL for *M. bovis* and *M. smegmatis*, respectively. A correlation between anti-tubercular and antioxidant (NO and DPPH) activities of the spices was found for *M. bovis* and *M. smegmatis* (R =0.8957, 0.8241; R= 0.7681, 0.7030,$p \leq 0.05$) respectively. The anti-tuberculosis properties of *Thonnigii sanguinea* and *Lepidum sativum* are being reported for the first time.

Keyword: Antioxidants, Anti-tuberculosis, Spices.

Introduction

Spices are usually processed plant substances used as additives for seasoning foods, to enhance taste, fragrance, flavour, colour, and preservation. These processed substances maybe flowers, seeds, fruits, leaves, barks, roots, rhizomes, and/or any other vegetative parts (Birt, 2006; Egharevba and Gamaniel, 2017). Spices are known to exhibit several physiologic properties in animals and humans and therefore are used in a number of medicinal preparations (Parthasarathy et al., 2008). Several researches

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have linked the prevention of several disease conditions to the consumption of some selected spices (Terry et al., 2001; Virgili et al., 2001; Farombi et al., 2011; Tende et al., 2015). The protective effects of spices are due to the presence of various bioactive molecules, which act solely or synergistically with one another. Some epidemiological and clinical studies have demonstrated a reduction in incidence of chronic and degenerative cardiovascular disease (CVD) in patient populations on high spice diets (Tende et al., 2015). Spices have been reported as a rich source of natural antioxidant molecules such as polyphenols, phenolic acids and their derivatives, tannins, flavonoids, phospholipids, ascorbic acid, carotenoids and sterols (Okwu, 2005). Due to the beneficial antioxidant properties of these spices, some have potentials for development as additives or adjuvant in formulation of medicines used for the management of chronic diseases like tuberculosis (TB), CVD and other degenerative oxidative stress diseases.

Tuberculosis is a killer infectious disease, and of serious public health concern in Nigeria. According to a report by WHO (2010), Africa had a TB incidence of 2.3 million and a prevalence of 2.8 million with about 250,000 recorded deaths in 2010; with, Nigeria ranked second among the 30 African countries with the highest burden of TB (WHO, 2017). Although recently, there has been a steady decline in TB incidences (Toyosi et al., 2018), and despite a 1.5% reduction in the incidence of TB between 2014 and 2015, the increase in new cases of multi drug-resistant tuberculosis has made TB treatment even more difficult. About, 480,000 new cases of multi-drug resistant TB were recorded in 2015 (WHO, 2016). Previous studies by Wild et al. (2004) have established that tissue inflammation, reduced dietary ingestion of micronutrients, the free radical surge from activated macrophages and anti-tuberculosis drug scan result in oxidative stress in tuberculosis patient. The free radicals generated in the process if not neutralized by antioxidants may contribute towards pulmonary inflammation. Anti-TB formulations with antioxidants found in spices could scavenge the free radicals generated in the process, in order to mitigate pulmonary inflammation.

In Nigeria, most of the commonly used spices grow in the wild, although a handful is cultivated. The majority of the spices available in Nigeria are found in the southern rainforest zone of the country, while others such as garlic and ginger are found mostly in the semiarid Northern zone. However, the antioxidant potential of some of these spices have not been assessed especially in consideration of their potential for use as an anti-TB adjuvant. This study aims to assess the comparative correlation between the phenolic contents, antioxidant, and anti-tubercular activities of some common Nigerian spices, which could be explored as the dietary supplement to enhance the management of tuberculosis or as a source of novel anti-TB drugs.

Materials and Methods

Equipment

The equipment used for the study include Agilent cary 60UV/Vis spectrophotometer and Memmertincubator KARL KOLB D-6072, Dreieich West Germany.

Chemicals and reagents

Ascorbic acid, 2,2-diphenyl-1-picryl hydrazyl (DPPH), quercetin, tannic acid, gallic acid, Griess reagent, and sodium nitroprusside were purchased from Sigma Aldrich Chemical Company, USA; Folin Ciocalteure agent was purchased from Loba Chemi ePvt Ltd, Mumbai, India. All solvents and other chemicals used for the study were of analytical grade.

Collection and extraction of spices

The spices (Table 1) were purchased from Karmo Market, Abuja Nigeria. They were pulverized and macerated in redistilled methanol for 72hr. The samples were filtered and the filtrates were concentrated to dryness under vacuum at 40^{o}C to obtain methanolic crude extracts.

Estimation of phenolic contents

Total phenolic contents

The spectrophotometric method previously reported by Adamu et al. (2018) was used on a concentration of 1 mg/mL extract. Briefly, 2.5mL of 10% Folin-Ciocalteure agent in water and 2.0 mL 7.5% Na_2CO_3 was added to 0.5 mL of the extracts in a test tube Blank was prepared using the equivalent volume of water in place of the extract. The reaction mixture was incubated in the dark for 30 min and absorbance was taken at 765 nm. This procedure was also done for the reference gallic acid standard and the calibration curve was plotted. The content of phenolic spresent in the extract was expressed as milligram of gallic acid equivalent per gram sample (mgGAE /g).

Total flavonoid contents (TFC)

The flavonoid content was determined according to the methods of Rebeya et al. (2015) with slight modifications. One mL of extract was added to 4 mL of distilled water in a test tube; 0.3 mL of 5 % sodium nitrite was

TABLE 1. List of spices used for the study.

Species	Family	Common name	Hausa name	Parts used
Zingiber officinale	*Zingiberaceae*	Ginger	Cittaraho	Rhizome
Syzgium aromaticum	*Myrtaceae*	Clove	Kanumfari	Flower buds
Cyperus articulatus	*Cyperaceae*	Piripiri	Kajiji	Seeds
Piper nigrum	*Piperaceae*	Black pepper	Masoro	Seeds
Thonningea sanguinea	*Balanophoraceae*	Ground pineapple	Kulla	Flower
Lepidum sativum	*Brassicaceae*	Garden cress seeds	Halim	Seeds
Zanthoxylum zanthoxyloides	*Rutaceae*	Sengal prick	Atar root	Stem bark
Xylopia aethiopica	*Annonaecae*	Negro pepper	Kimba	Whole fruit
Nigella sativa	*Ranunculaceae*	Black seeds/Black cumin		Seeds

added and incubated for 5 min.0.3 mL of 10 % aluminum chloride was added to the mixture and incubated for 5 min. Two mL of 1M sodium hydroxide was added to the tube and made up to 10mLwith distilled water. Quercetin was used as the reference drug. Absorbance was measured at 510nm. A calibration curve was constructed and used for the quantification of flavonoid in the extracts. The flavonoid content was expressed as milligram of quercetin equivalent per gram sample (mgQE/g).

Estimation of total tannins

The content of tannin was determined using Folin-Ciocalteu assayas described by Mesfin and Won (2019) with some modifications.0.25mL of Folin-Ciocalteu reagent and 1.25 mL of Na_2CO_3 (10 % *w*/*v*) was added to 0.5mL of extract and then incubated for 40 min. Tannic acid was used as a standard and absorbance was taken at 725nm. The results were presented in mgTAE/g.

Antioxidant assays

DPPH free radical scavenging activity

The free radical scavenging ability of the extracts was evaluated *in vitro* using 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay as previously reported by Chinaka et al. (2013). DPPH radical scavenging assay is centered on the capacity of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The presence of an unpaired electron in DPPH radical is responsible for the visible deep purple colour and the absorbance at 517nm. When DPPH accepts an electron from an antioxidant, the effect can be measured quantitatively from the changes in absorbance (Saha et al., 2018). Briefly, 1.0 mL of 0.3 mM DPPH in methanol was added to 1.0 mL of different concentrations of the extract (400-25µg/mL) and kept in the dark for 30 min. Ascorbic acid was used as a reference antioxidant. The absorbance was measured at 517nm, and scavenging activity was expressed as the percentage of DPPH radical scavenged, using the following equation:

$$\% \text{ inhibition} = [1-(A_1 / A_0) \text{ X } 100]$$

Where A_1 is the absorbance of the extracts and A_0 is the absorbance of the control.

IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated.

Nitric oxide inhibitory assay

The ability of the extracts to inhibit nitric oxide was evaluated using Greiss reaction method as previously reported by Jagetia et al. (2004). 10mM sodium nitroprusside in phosphate-buffered saline pH (7.4) was mixed with different concentrations of the extract in a test tube and incubated at 25°C for 180min. 1 mL of Greiss reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) was reacted with 1mL of the mixture and the absorbance was read at 550 nm. Inhibitory actions of nitrite formation by the extract and the standard antioxidant ascorbic acid were calculated relative to the control. IC_{50} was determined.

Anti-tubercular study

Culture and growth of mycobacterium

American typed cultures of *Mycobacterium bovis* (27290) and *Mycobacterium smegmatis* (607) were used for this study. The test organisms were inoculated into 7H9 Middle brook both supplemented with Albumin Dextrose Complex (ADC) and incubated at 37°C for 5-7 days. The grown cultures had it soptical density adjusted to between 0.2-0.3 on a UV-Visible (Agilent cary 60) spectrophotomete rat a wave length of 650nm. The organisms were diluted further by diluting 50 µL of the organism into 50 mLof 7H9/ADC broth (i.e a dilution ratio of 1:1000) for the study.

Susceptibility study against M. bovis and M. smegmatis

The susceptibility test of the spices was conducted using the broth micro-dilution technique in a 96 well micro-titre plate (Clinical Laboratory Standards Institute (CLSI), 2012). Briefly, 100 mg/mL extract concentration was prepared by dissolving 100 mg of each spice extract in 0.2 mL dimethylsulfoxide (DMSO) and made up with 0.8 mL of sterile water. The Control drug isoniazid (Sigma Aldrich Inc) was prepared by dissolving 25 mg of the drug in 1 mL DMSO, and 25 µg/mL solution was made by diluting 25 µL in 25 mL 7H9/ADC broth, sterile filtered. 50 µL of sterile 7H9/ADC broth was dispensed into the 96 well micro-titre plates, 50 µL of 100 mg/mL concentration of the stock sample was transferred into well 1 of the plate. 50 µL of the mixed solution in well 1 was transferred into well 2, mixed thoroughly and repeated through to well 11Well 12 was used asthe organism viability control (OVC). All the wells (1-12) were inoculated with 50 µL of the diluted culture and incubated at 37°C for 5-7 days. Control wells with isoniazid ranging from 12.5-0.02 µg/mL were also carried out. After incubation, 25 µL of tetrazolium salt dye was added to all the wells, re-incubated for 24 hr and observed for the absence or presence of microbial growth by the color change in the wells. The colorless well was interpreted as no growth of test organism and pink color was interpreted as growth occurrence. Minimum inhibition concentration (MIC) was taken as the lowest concentration of drug/extract that prevented color change.

Statistical analysis

All analysis was carried out in triplicate. The results were calculated and analysed using Graph Pad® Prism Software version 6. The results were expressed as mean ± standard deviation (SD) and also subjected to analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Correlation between phenolics, flavonoids and tannins content, antioxidant and anti-tubercular activities was determined using Pearson's correlation. p values 0.05 were regarded as significant.

Results

Total phenolic content

The total phenolic contents of the extracts of the different spices were determined from a linearregression curve ($y = 0.0086x + 0.0602$, $R^2 = 0.9678$) of Gallic acid, and expressed as milligram of gallic acid equivalents per gramsample (mgGAE/g sample). The results, as presented in Table 2, showed that the extracts had phenolic content ranging from 7.3 – 193.9 mgGAE/g. *S. aromaticum and T. sanguinea* had the highest phenolic content of 193.9 and 122.5 mgGAE/g, respectively, while *X. aethiopica* extrac thad the least phenolic content of 7.3 mgGAE/g.

Total flavonoid content (TFC)

The total flavonoid contents of the extracts were estimated from a linear regression curve ($y = 0.0034x - 0.0386$, $R^2 = 0.9749$) of Quercetin, a standard flavonoid, and presented in milligram quercetin equivalents per gram sample (mgQE/g). Table 2 showed that the extracts had flavonoid content ranging from 0 – 12.59 mgQE/g.*C. artikulatus* and *S. aromaticum* had 12.59 & 10.79 mgQE/g while *L. sativum* and *X. aethiopica* had 0 (zero) flavonoid content.

Total tannin content

The tannin contentsof the extracts were estimated from a regression curve ($y = 0.001x + 0.0465$, $R^2 = 0.9387$) of tannic acid. The resultsshowed that the extracts had significant levels of tannin ranging from 0 – 44.4 mgTAE/g. *S. aromaticum* and *Z. officinale* had 44.4 & 25.0mgTAE/g while tannins were not detected in *N. sativa* and *X. aethiopica* (Table 2).

Radical scavenging assays

In this assay, the investigated spices had various degrees of DPPH radical inhibition. The results indicated that *S. aromaticum* was the most active with IC_{50} value of 4.2µg/mL while *N. sativa* was the least active of IC_{50} value1098.0 µg/mL. The extracts also exhibited NO inhibitory activities. *F. zanthoxyloides* and *S. aromaticum* had IC_{50} values of 0.0025 and 0.0051µg/mL which were comparable to that of gallic acid which had a value of 0.0008µg/mL (Table 3).

Anti-tuberculosis activity

The results showed that *M. bovis* and *M. smegmatis* were susceptible to the spices with varying degrees of anti-tuberculosis activities. The spices MICs against *M. bovis* and *M. smegmatis* ranged from 391 - 25000 µg/mL and 781 - 12500 µg/mL, respectively. *L. septivum* and *T. sanguinea* were the most active, inhibiting the growth of *M. bovis* and *M. smegmatis* with MICs of 391µg/mL and 781µg/mL, respectively, whereas *N.sativa* was less effective against both organisms (Table 4).

Discussion

Antioxidants activities

Antioxidants are believed to exert their actions through mechanisms such as radical scavenging, reducing capacity, metal chelating and decomposition of peroxides (Cook and Samma, 1996). Thus, antioxidant capacities differ depending on the assay used for assessment (Yen et al., 2005). Therefore, the antioxidant activities in this study were evaluated using different models.

TABLE 2. Phenolic, Flavonoid and Tannin Contents of the spices.

Extracts/ Control	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QE/g)	Total condensed tannin content (mg TAE/g)
S. aromaticum	193.9 ±0.^{2a}-i	10.7 ±0.5^{b-i}	44.4 ±0.3^{a-i}
Z. officinale	53.4 ±0.1^{a-i}	7.9 ±0.6^{a-i}	25.0 ±0.6^{a-i}
X. aethiopica	7.3 ±0.1^{a-i}	0.0±0.0^{a,b,d,e,h}	0.0±0.0$^{a,b,d,e,g-i}$
P. nigrum	47.1 ±0.4^{a-i}	0.9 ± 0.2$^{a-c,e,g,h}$	13. 1 ± 0.3^{a-i}
T. sanguinea	122.5 ±0.7^{a-i}	1.7± 0.1$^{a-d,f,g-i}$	44.1 ±0.5^{a-i}
N.sativa	9.9 ± 0.1^{a-i}	0.4±0.0^{a,b,e,h}	0.0±0.0$^{a,b,d-i}$
L.sativum	22.0 ±0.1^{a-i}	0.0±0.0^{a,b,d,e,h}	9.3 ±0.2^{a-i}
C.articulatus	30.9 ±0.0^{a-i}	12.5± 0.2$^{a-f,g,i}$	9.0±0.4^{a-i}
Z. zanthoxylides	22.3 ±0.3$^{a-f,h}$	0.08± 0.0$^{a-f,h}$	17.9±0.1^{a-i}

Key: Means with different superscripts within each column differ significantly ($p \leq 0.05$) from one another**.**

TABLE 3. Antioxidant activities of spices.

Extracts / Reference	DPPH Free Radical Scavenging Activity IC_{50} (µg/mL)	Nitric Oxide Inhibition IC_{50} (µg/mL)
S. aromaticum	4.2^{b-i}	0.0051^{c,e,f}
Z. officinale	20.8$^{a,c-i}$	0.55^{c,e,f}
X. aethiopica	321.7$^{a,b,d-i}$	28.65$^{a,b,d-i}$
P.nigrum	41.7 $^{a-c,e-i}$	0.32^{c,e,f}
T. saguinea	7.7$^{a-d,f-i}$	6.20^{a-i}
N.sativa	1098.0$^{a-e,g-i}$	47.48^{a-i}
L. sativum	107.0$^{a-f,h,i}$	0.57^{c,e,f}
C. articulatus	33.8 $^{a-g,i}$	0.81^{c,e,f}
Z. zanthoxyloides	32.8^{a-h}	0.0025^{c,e,f}
Ascorbic acid	3.6*	-
Gallic acid	-	0.00088*

Key: *significantly different ($p \leq 0.05$) from extracts, Means with different superscripts within each column differ significantly ($p \leq 0.05$) from one another.

TABLE 4. Anti-tubercular activity (µg/mL)of spices against *M. bovis*and *M. smegmatis*.

	Spices/Control	*M. bovis*	*M. smegmatis*
1	*S. aromaticum*	6250$^{c\text{-}g,i}$	6250$^{c\text{-}g,i}$
2	*Z. officinale*	6250$^{c\text{-}g,i}$	3125$^{b,e\text{-}h}$
3	*X. aethiopica*	3125 $^{a,b,e\text{-}h}$	6250 $^{c\text{-}g,i}$
4	*P.nigrum*	3125$^{b,e\text{-}h}$	6250$^{c\text{-}g,i}$
5	*T. saguinea*	781 $^{a\text{-}d,f\text{-}i}$	781$^{a\text{-}d,f\text{-}i}$
6	*N.sativa*	25000 $^{a\text{-}e,g\text{-}i}$	12500$^{a\text{-}e,g\text{-}i}$
7	*L. sativum*	391$^{a\text{-}i}$	6250$^{a\text{-}d,f,h,i}$
8	*C. articulatus*	6250$^{c\text{-}g,i}$	6250 $^{a\text{-}d,f,g\text{-}i}$
9	*Z. zanthoxyloides*	3125$^{a,b,e\text{-}h}$	3125$^{a,b,e\text{-}h}$
10	Isoniazid	0.02*	0.02*

Key: *significantly different (p0.05) from extracts, Means with different superscripts within each column differ significantly (p 0.05) from one another.

The extracts showed various degrees of DPPH radical scavenging activities with *S. aromaticum* and *T. sanguinea* having the highest DPPH scavenging activities with $IC_{50}$of 4.2 and 7.7µg/mL, respectively, which were comparable to that of ascorbic acid (3.6µg/mL).

Z. zanthoxyloides and *S. aromaticum* also exhibited significant NO inhibitory activities (p0.05) with IC_{50} of 0.0025 and 0.0051 µg/mL, respectively, which is comparable to gallic acid (0.00088µg/mL). Sıowianek and Leszczyska (2016) reported that *S. aromaticum* had the highest antioxidant activities in their studies. Several studies had also indicated that *S.aromaticum* have very good antioxidant activity and high levels of phenolics (Gülçin et al., 2004; Shan et al., 2005; Kim et al., 2011; Ouattara et al., 2013). Nitric oxide (NO) is a free radical that plays role in mediating several physiological functions such as vascular homeostasis, neurotransmission, antimicrobial defense and antitumor activities (Rozina et al., 2013). However, at high concentrations, Nitric oxide (NO) has been reported to play adverse role in inflammation, cancer, non-specific host defense and several disease state (Rozinal et al., 2013, Adamu et al., 2018). Perhaps, an adjuvant combination of *S. aromaticum*, *T. sanguinea* and *Z zanthoxyloides* could be relevant as a strong antioxidant base combination in anti-TB formulations.

Antioxidant activities in substances from plant origin have been strongly related to the relative abundance of phenols and flavonoids. Natural phenolics include simple compounds, such as phenolic acids and extremely polymerized compounds such as tannins (Ayalew et al., 2016). Phenolic compounds are diverse and well known for their antioxidant and radical scavenging properties (Edak et al., 2013). The antioxidant ability of phenols is understood to be due to the hydroxyl group (-OH) that is bonded directly to an aromatic hydrocarbon (phenyl) ring in the structure. This makes them donate electrons readily to electron-seeking free radicals, thus preventing their hazards in living cells. Flavonoids are the most popular and dispersed collections of plant phenolics. They are soluble in water and play a role in scavenging free radicals, inhibition of peroxidation and chelating transition metals (Nikavar et al., 2007). Tannins are water-soluble phenolic compounds with antioxidant and antimicrobial properties. Their actions are associated with the inhibition of lipid peroxidation free radical scavenging (Okuda, 2005, 2011). High antioxidant and free radical scavenging activities may be as a result of the high level of phenols and flavonoids present in the extracts (Cook and Samman, 1996).

Anti-tubercular Activity

Previously studies have shown that spices like *Allium sativum* (Fam. Liliaceae), *Foeiniculumvulgare* (Fam. Apiaceae), *Curcuma longa* (Fam. Zingiberaceae) *Juniperus excelsa* (Fam. Cupressaceae), *Trachyspermum ammi* (Fam. Apiaceae), *Piper longum*, *Piper nigrum* (Fam. Piperaceae) and *Nigella sativa* (Fam.

Ranunculaceae) are among the most active against tuberculosis causing pathogens. This research investigated *piper nigrum, N. sativa* and other indigenous spices for their antituberculosis activity against *M. bovis* and *M. smegmatis*. A study by Joseph et al. (2018), reported the anti-tubercular activity of *N. sativa* seed extract against *M.tuberculosis* H37Rv, all drug-sensitive *M. tuberculosis,* and MDR *M. tuberculosis*, respectively at different concentrations. Similarly, Forouzanfa et al. (2014), reported that different extracts of *N. sativa*, as well as Thymoquinone (TQ), have broad antimicrobial activity against both Gram-negative, Gram-positive bacteria. Thymoquinone (TQ) has been considered as one of the most active constituents present in *N. sativa* seeds and possesses anti-TB activity at 20 µg/mL concentration (Joseph et al., 2018). The research reported by Sivakumar and Jayaraman, (2011), showed that the ethanolic extract of *S. aromaticum* had a MIC of 200µg/mL against *M. tuberculosis* H37Ra. The study of Joseph et al**.**(2016) showed that the hydro-ethanolic extract of *Z.officinale* was able to inhibit the growth of the *M. tuberculosis* strain H37Ra and *M. smegmatis* with MICs of 2500 µg/mL and 10000 µg/mL respectively which is in agreement with the present study. In another study by Benjamin et al. (2014), *M. abscessus and M. fortuitum* was susceptible to *Z.officinale* extract with a MIC of 6250 µg/mL, however, it was not effective against *M. smegmatis* at the tested concentrations. Patilaya et al.(2012) reported that the ethanol extract of *P. nigrum* exhibited the best activity against *M. tuberculosis* with a MIC value of 100µg/mL while the ethylacetate, n-hexane, and the water fractions of *P.nigrum* showed antimycobacterial activity against *M. tuberculosis* with MIC values of 25, 50, and 100 µg/mL respectively. Similarly, Deepthi et al. (2012) reported the excellent antimycobacterial activity of piperine, a major compound found in *P. nigrum*. Piperine, is a trans-trans isomer of 1-piperonyl-piperidine, an antimycobacterial agent which at 128µg/mL completely inhibits the efflux pump of *M. smegmatis* (Jin et al., 2011). This compound is commonly found in the family Piperaceae to which *P. nigrum*belongs. Investigations on ginger rhizome (*Z. officinale*) revealed that the phenolics,6-gingerol, 8-gingerol and 10-gingerol. 8-gingerol and 10-gingerol were found to be more active against *M. tuberculosis* H37Rv28, with MIC values of 25-50 µg/mL.

In the present study, a significant correlation between anti-tubercular and DPPH antioxidant activities of the spices was found for *M. bovis* and *M. segmantis* (R =0.8957, 0.8241; p 0.05), respectively, although the anti-TB activities were generally weak (Table 4). A lower correlation between anti-tubercular and NO inhibitory activities (R = 0.7681, 0.7030; p≤0.05) for *M. bovis* and *M. segmantis*, respectively was also observed. This generally implies that there is a significant relationship between the anti-tubercular and DPPH/NO antioxidant properties. Several studies have reported the relationship between the antioxidant activities of phenolic compounds as hydrogen donors and free radical scavengers. Khlifi et al.(2011) reported a good correlation between the polyphenols contents or flavonoids content and antioxidant activity of different extracts of *G. alypum*. In this study,there was no correlation between anti-tubercular activity, phenol, flavonoid and tannin contents of the spices. This is in agreement with the finding of Khlifi et al. (2011), where there was no correlation between anti-tuberculosis activity and the total phenolic content. The marginal anti-TB activity and zero correlation between anti-TB and phenolic content possibly suggest that the antioxidant activities of the phenolics may be countered by some other secondary metabolites that may be present in the extract.

Wiid et al. (2004), proposed that the total antioxidant status of TB patients should be measured for more effective disease control because diets low in antioxidants may render individuals susceptible to tuberculosis. This is because pulmonary tuberculosis has been found in patients with an increased level of free radicals. The presence of free radicals during active disease contributes towards the prevalent tissue damage seen in TB cases and thus indicates a need for dietary supplementation for improved healing and rapid recovery. An improved total antioxidant status of TB patients would either be as a result of the defensive effect of antioxidants rich diet supplement and/or a reduced free radicals generation. Spices could also be considered as a natural source of antioxidants, therefore an increase in spices intake could help in the management of TB.

Conclusion

The findings of the present study indicate that the evaluated spices are rich in phenols, tannins, and flavonoids; in addition, they possess a varying degree of antioxidant and anti-tubercular activities. The correlation between antioxidant and antituberculosis activities is reported for the first time in these spices. The correlation was found between free-radical scavenging antioxidants of the spices investigated and antituberculosis activities. But there was no correlation between the polyphenol contents and anti-TB. The spices may be acting mainly through free-radical scavenging. This study has shown that spices could be harnessed as dietary supplementation for improved healing and rapid recovery in TB patients.

Conflict of Interest

The authors declare that they have no competing interests.

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