

TOTAL PHENOLIC CONTENT AND FREE RADICAL SCAVENGING ACTIVITY OF REPRESENTATIVE MEDICINAL PLANTS OF THAILAND

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ABSTRACT

Objective: Plants are the vital source of pharmaceutically important compounds with less or no adverse side effects. The current study was conducted to catalog the commonly used indigenous and medicinal plants of Thailand based on their phenolic acid content and antioxidant activity.

Methods: The herbs were collected from Chiang Mai province, Thailand. The plants were extracted with 70% ethanol. The total phenolic acid content and antioxidant activity were evaluated.

Results: The ethanolic extract of plant samples was prepared. Among the tested plant samples, *Phyllanthus emblica* Linn. and *Terminalia bellerica* Roxb. showed highest phenolic content (Gallic acid equivalent [GAE]; 764.81 mg GAE/g sample) and antioxidant activity (trolox equivalent antioxidant capacity [TEAC]; 394.20 mg/g sample), respectively. About 94-97% of inhibition of free radical was detected in 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay tested against the plant samples. The ethanolic extract of *Anaxagorea luzonensis* Gray., *Terminalia* sp., *T. bellerica* Roxb., *Terminalia chebula* Retz., *Albizia procera* Benth., *Harrisonia perforata* Merr., and *P. emblica* Linn. exhibited 97.87, 96.08, 92.26, 86.74, 86.08, 84.47, and 83.13% of superoxide radical inhibition, respectively.

Conclusion: The results suggested that *T. bellerica* Roxb. possessed high TEAC ability and DPPH radical scavenging capacity and *A. luzonensis* Gray. exhibited high superoxide scavenging activity, when compared to that of the other tested samples. The additional detailed study is desirable to understand the complexity and distribution of bioactive compounds present in the commonly used plant species of Thailand.

Keywords: Antioxidant activity, Ethanolic extract, Phenolic acids, Thai plants.

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INTRODUCTION

Plants are the rich source of vitamins, minerals, and other essential nutrients. Most of the plants have a medicinal property which is attributed to the high content of phenolic acids, flavonoids, and other phytochemicals. The use of plant and plant derivatives as medicine for the prevention and treatment of several diseases is recorded for thousands of years, and it is the ancient practice to cure the diseases. The key mechanism of healing ability of the medicinal plant and its phytochemicals is free radical scavenging activity.

Numerous studies have reported the medicinal uses of several plant species [1,2]. The antioxidant ability of Indian medicinal herbs has been reviewed. Some of the plants such as *Amaranthus paniculatus*, *Aerva lanata*, *Coccinia indica*, and *Coriandrum sativum*, which are rich in antioxidant compounds, are used in regular cooking as vegetables showing that these plants could be a source of dietary antioxidants [3]. Krishnaiah *et al.* [4] reviewed about the antioxidant potential of medicinal plant species such as *Pistacia lentiscus*, *Diospyros abyssinica*, *Sargentodoxa cuneata* Rehd. Et Wils, *Crataeva nurvala* Buch-Ham., *Polyalthia cerasoides* (Roxb.) Bedd., *Acacia auriculiformis* A. Cunn., *Momordica charantia* L., *Rheum ribes* L., *Teucrium polium* L., *Urtica dioica* L., *Bidens pilosa* Linn. *Radiata*, *Ficus microcarpa* L. fil., *Leea indica*, *Uncaria tomentosa* (Willd.) DC, *Geranium sanguineum* L., *Dracocephalum moldavica* L., *Salvia officinalis* L., and *Pelargonium endlicherianum*. The studies revealed that the natural antioxidants are effective, and side effect-free substitute to synthetic compounds in the functional food productions, as well as in medicine.

Some of the reports explained about the antioxidant activity and antioxidant compounds of edible plants and commonly found plants of Thailand [5,6]. The documentation of antioxidant capacity and other related phytochemical contents of the frequently used plants are essential to explore the naturally available medicinal compounds. Thus, the purpose of this study was to document the total phenolic acid content and antioxidant ability of the most generally used indigenous and medicinal plants of Thailand, collected in the region of Chiang Mai.

METHODS

Sample collection and extraction

The plant samples were collected at local markets of Chiang Mai, Thailand, and the plant species were confirmed by comparing with the herbarium specimen of Faculty of Pharmacy, Chiang Mai University, Thailand.

The collected plant samples were cleaned with sterile water and smashed into small pieces. About 100-200 g of plant samples were mixed with 400-700 ml of ethanol:water (7:3) solvent system, and the samples were heated and extracted using Soxhlet extractor. For the indigenous plant samples, the extraction was done without heating step and without using soxhlet extractor. Indigenous plant samples were soaked in prescribed amount of 70% ethanol for 1 hr. Then, the extracts were filtered through Whatman No. 1 filter paper, and the extracts were collected and stored in an amber bottle at 4°C until use.

Total phenolic content

The total phenolic content of the extracted plant samples was determined as described in the previous studies [7], and the total phenolic content was represented as mg of gallic acid equivalent (GAE) (mg GAE) per g of plant extract.

Free radical scavenging activity

Total antioxidant capacity of the tested plant extracts was evaluated by 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assay as described previously [7], and the results were represented as Trolox equivalent antioxidant capacity (TEAC) per g of the plant extract. In addition, the free radical scavenging property of the plant extracts was also evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and superoxide anion radical scavenging assay as detailed previously [7,8]. Both DPPH and superoxide anion radical scavenging assay results were represented as a percentage of inhibition. All the samples were tested in triplicate.

Statistical analysis

The data were represented as mean±SD. ANOVA with least significant difference *post-hoc* test was executed to evaluate the significant differences and *p*<0.05 was considered to be statistically significant using statistical SPSS software version 16 (Chicago, SPSS Inc., U.S.A).

RESULTS AND DISCUSSION

The plant samples were collected (Table 1) and the contents were extracted with ethanol:water (7:3 ratio) extraction method. Then, the extracts were subjected to total phenolic acid content analysis by Folin-Ciocalteu method and free radical scavenging activity by ABTS, DPPH, and superoxide anion radical scavenging assays.

The phenolic acid content of the ethanolic extract of the tested plant samples was represented as GAE (mg/g extract). The top 10 plant species with highest GAE values are as follows *Phyllanthus emblica*

Linn. (764.81 mg/g extract) >*Betula alnoides* Buch Ham. (620.80 mg/g extract) >*Terminalia* sp. (506.41 mg/g extract) >*Anaxagorea luzonensis* Gray. (438.03 mg/g extract) >*Terminalia chebula* Retz. (389.18 mg/g extract) >*Caesalpinia mimosoides* Lamk. (234.92 mg/g extract) >*Jussiaea repens* Linn. (229.41 mg/g extract) >*Gymnema inodorum* Decne. (83.35 mg/g extract) >*Manihot esculenta* Crantz. (75.78 mg/g extract) >*Ocimum sanctum* Linn. (62.39 mg/g extract). The least amount of total phenolic acid content was detected in the ethanolic extract of *Spilanthes acmella* (Linn.) Murr. (1.41 mg/g extract) (Fig. 1).

The total phenolic content, antioxidant property, antimutagenic, and antibacterial activities of ethanolic extract of *P. emblica* branches were comparable to the ability of *P. emblica* fruits [9]. The polyphenolic content of *P. emblica* fruit was enriched during the fermentation with *Lactobacillus paracasei* H1101 [10]. *T. chebula* has been reported to contain gallic acid, ellagic acid, tannic acid (about 30%), ethyl gallate, chebulic acid, chebulagic acid, corilagin, mannitol, ascorbic acid, and other compounds [11,12]. The species of *Caesalpinia* are accounted for its phytochemical contents and pharmacological importance and are evidently reviewed by Zanin *et al.* [13].

M. esculenta Crantz. leaves extract was reported for phenols, anthocyanins, and ascorbic acid content with free radical scavenging properties [14]. Coniferaldehyde, isovanillin, 6-deoxyjacareubin, scopoletin, syringaldehyde, pinoresinol, p-coumaric acid, ficusol, balanophonin, and ethamivan are the known antioxidant compounds present in *M. esculenta* Crantz. [15]. About 254.44 TAE/mg of extract of tannin, Vitamin C (1.7-419 mg/100 g), β -carotene (around 23-86 mg/100 g), Vitamin A, saponins, steroids, and glycosides were reported in *M. esculenta* Crantz. [16-18].

O. sanctum Linn. is made of multiple chemical constituents, and the composition of the phytochemical was varied among the cultivars and affected by the processing, storage, etc., *O. sanctum* Linn. oil contains

Table 1: List of plants and plant parts collected and used in the study

Sample No.	Indigenous plants	Part used	Sample No.	Medicinal plants	Part used
1	<i>Ocimum sanctum</i> Linn.	Fresh leaf	16	<i>Betula alnoides</i> Buch Ham.	Wood
2	<i>Caesalpinia mimosoides</i> Lamk.	Apical bud	17	<i>Anaxagorea luzonensis</i> Gray.	Stem
3	<i>Piper sarmentosum</i> Roxb.	Fresh leaf	18	<i>Betula alnoides</i> Buch Ham.	Core of stem
4	<i>Plantago major</i> Linn.	Whole plant	19	<i>Streblus asper</i> Lour.	Stem
5	<i>Monochoria vaginalis</i> Presl.	Fresh leaf	20	<i>Harrisonia perforata</i> Merr.	Root
6	<i>Spilanthes acmella</i> (Linn.) Murr.	Whole plant	21	<i>Plumbago indica</i> Linn.	Root
7	<i>Gymnema inodorum</i> Decne.	Fresh leaf	22	<i>Capparis micracantha</i> DC.	Root
8	<i>Polygonum flaccidum</i> Meissn.	Whole plant	23	<i>Albizia procera</i> Benth.	Stem bark
9	<i>Iresine herbstii</i> Hook.	Fresh leaf	24	<i>Clerodendrum indicum</i> Kuntze.	Root
10	<i>Oenanthe javanica</i> DC.	Fresh leaf	25	<i>Phyllanthus emblica</i> Linn.	Fruit
11	<i>Oroxylum indicum</i> Vent.	Root	26	<i>Ficus racemosa</i> L.	Fruit
12	<i>Jussiaea repens</i> Linn.	Fresh leaf	27	<i>Tiliacora triandra</i> Diels.	Fresh leaf
13	<i>Antidesma ghaesembilla</i> Gaertn.	Fruit	28	<i>Terminalia chebula</i> Retz.	Dry fruit
14	<i>Manihot esculenta</i> Crantz.	Apical bud	29	<i>Terminalia</i> sp.	Dry fruit
15	<i>Piper ribesoides</i>	Fresh leaf	30	<i>Terminalia belerica</i> Roxb.	Dry fruit

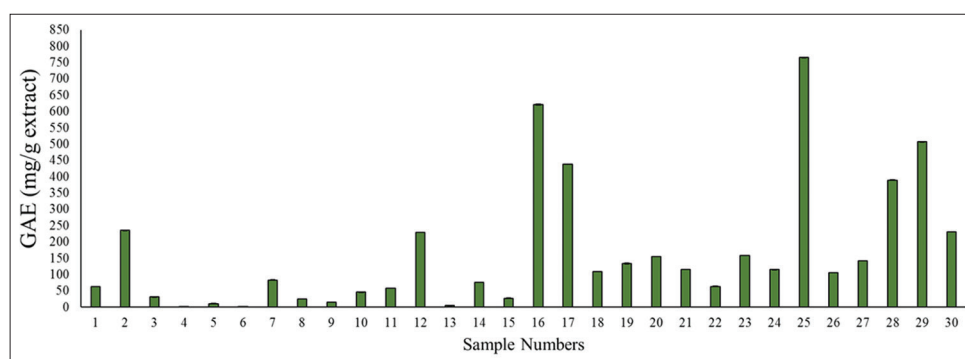


Fig. 1: The total phenolic content (Gallic acid equivalent) of the plant extracts

eugenol, euginal, ursolic acid, carboxylic acid, carvacrol, linalool, limatrol, caryophyllene, methyl carvicol, fatty acids, and sitosterol. The chemical constituents of *O. sanctum* were detailed clearly by Pattanayak et al. [19].

The current study suggested that *P. emblica* Linn. had high phenolic content (764.81 mg/g sample) when compared to that of the other tested samples (Fig. 1).

Total antioxidant capacity of the plant extracts was determined and represented as TEAC (mg/g sample). The top 10 plant samples with highest TEAC values are as follows: *Terminalia bellerica* Roxb. (394.20 mg/g extract) > *P. emblica* Linn. (392.98 mg/g extract) > *Terminalia* sp. (390.77 mg/g extract) > *T. chebula* Retz. (388.95 mg/g extract) > *A. luzonensis* Gray. (385.41 mg/g extract) > *Caesalpinia mimosoides* Lamk. (196.15 mg/g extract) > *J. repens* Linn. (194.21 mg/g extract) > *Polygonum flaccidum* Meissn. (190.31 mg/g extract) > *Antidesma ghaesembilla* Gaertn. (154.72 mg/g extract) > *Piper sarmentosum* Roxb. (153.56 mg/g extract). The least value of TEAC

acid was recorded in the ethanolic extract of *Tiliacora triandra* Diels. (45.80 mg/g extract) (Fig. 2).

In DPPH assay, about 94-97% of inhibition was found to be exhibited by the tested plant extracts. More specifically, *T. bellerica* Roxb. (97.47±0.06%), *C. mimosoides* Lamk. (97.20±0.08%), *P. emblica* Linn. (97.17±0.01%), *Terminalia* sp., (96.62%), *J. repens* Linn. (96.24±0.02%), *T. chebula* Retz. (96.17±0.01%), *A. luzonensis* Gray. (95.30±0.08%), and *P. flaccidum* Meissn. (94.30±0.05%) showed high activity in DPPH assay. Whereas, *Streblus asper* Lour. (2.98±0.37%), *Capparis micracantha* DC. (2.62±0.26%), and *Monochoria vaginalis* Presl. (2.15±1.1%) displayed a non-significant level of activity in DPPH assay (Fig. 3).

The superoxide radical scavenging activity of the tested extracts was determined. The ethanolic extract of *A. luzonensis* Gray., *Terminalia* sp., *T. bellerica* Roxb., *T. chebula* Retz., *Albizia procera* Benth., *Harrisonia perforata* Merr., and *P. emblica* Linn. showed 97.87, 96.08, 92.26, 86.74, 86.08, 84.47, and 83.13% of superoxide radical inhibition, respectively. The minimum superoxide radical inhibition activity was observed in

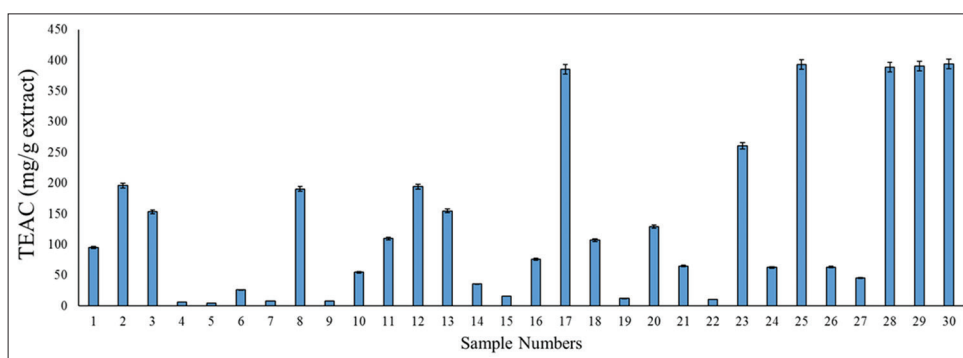


Fig. 2: The antioxidant capacity (trolox equivalent of antioxidant capacity) of the plant extracts

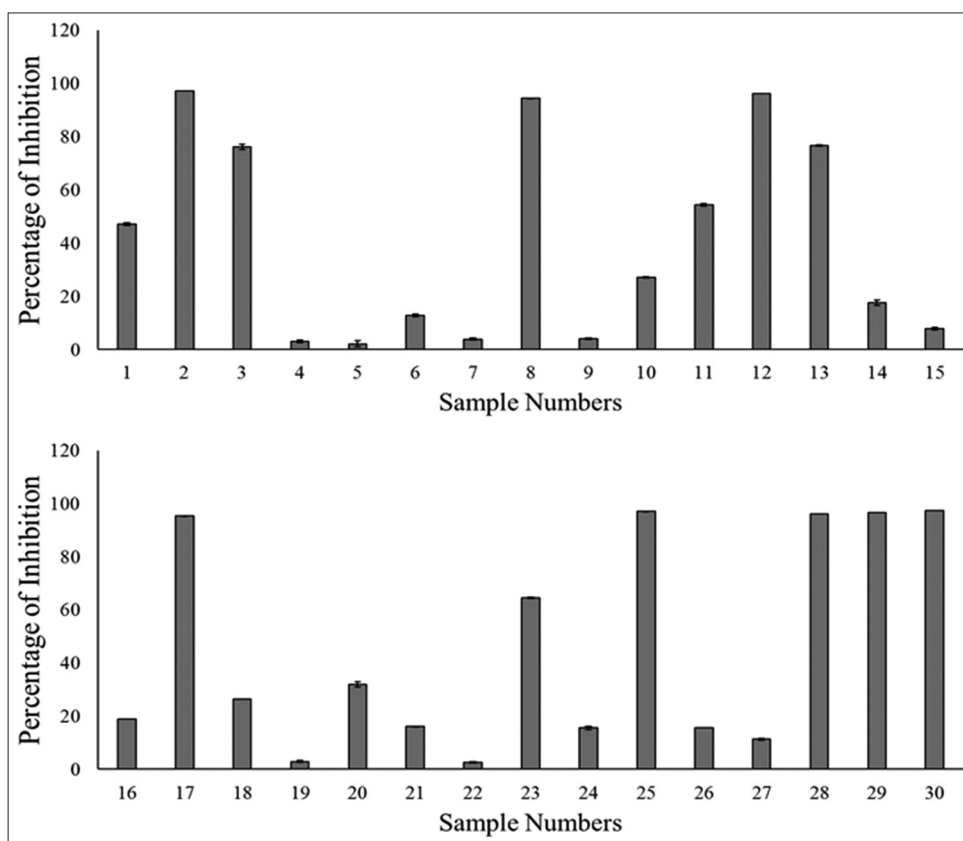


Fig. 3: Percentage of inhibition of free radical by plant extracts in 2,2-diphenyl-1-picrylhydrazyl assay

Table 2: Categorization of top five potent plant species based on the total phenolic acid content and antioxidant activity

Rank	Phenolic content (GAE)	Total antioxidant capacity (TEAC)	% of inhibition (DPPH assay)	% of inhibition (superoxide radical assay)
1	<i>Phyllanthus emblica</i> Linn.	<i>Terminalia belerica</i> Roxb.	<i>Terminalia belerica</i> Roxb.	<i>Anaxagorea luzonensis</i> Gray.
2	<i>Betula alnoides</i> Buch Ham.	<i>Phyllanthus emblica</i> Linn.	<i>Phyllanthus emblica</i> Linn.	<i>Terminalia</i> sp.
3	<i>Terminalia</i> sp.	<i>Terminalia</i> sp.	<i>Terminalia</i> sp.	<i>Terminalia belerica</i> Roxb.
4	<i>Anaxagorea luzonensis</i> Gray.	<i>Terminalia chebula</i> Retz.	<i>Terminalia chebula</i> Retz.	<i>Terminalia chebula</i> Retz.
5	<i>Terminalia chebula</i> Retz.	<i>Anaxagorea luzonensis</i> Gray.	<i>Anaxagorea luzonensis</i> Gray.	<i>Phyllanthus emblica</i> Linn.

GAE: Gallic acid equivalent, TEAC: Trolox equivalent antioxidant capacity, DPPH: 2,2-diphenyl-1-picrylhydrazyl

the *M. vaginalis* Presl. (4.83%) and *Oenanthe javanica* DC. (7.38 %) extract (Fig. 4).

The literature survey suggested that the fruits of *P. emblica* Linn. have been reported for its antioxidant, antipyretic, antimicrobial, anticancer, anti-inflammatory, immunomodulatory, hypolipidemic, and hepatoprotective properties [20-27]. The methanolic extract of *P. emblica* has been reported for the bacterial efflux pump inhibiting activity [28]. The aqueous extract of *P. emblica* exhibited the antidepressant activity at higher doses in mice [29].

The dried ripe fruit of *T. chebula* is an essential herb used widely in the indigenous system of medicine for its homeostatic, laxative, antitussive, diuretic, and cardiotoxic activities [30].

Caesalpinia sp. extract has antiulcer, anticancer, antidiabetic, anti-inflammatory, antimicrobial, and antirheumatic properties [13,31].

The alcoholic extracts of *Jussiaea* species were stated to have antibacterial, anti-inflammatory, antidiarrheal, antitumor, antitussive, and antipyretic activities [32]. Ethyl acetate extract of aerial parts of *J. repens* was non-toxic and exhibited antioxidant, anti-inflammatory, hepatoprotective, and antidiabetic activities [32]. *G. inodorum* has been reported for hypoglycemic effect in healthy human [33].

M. esculenta Crantz. has been used to treat fever, diarrhea, headache, rheumatism conjunctivitis, abscess, demulcent, hypertension, marasmus, irritable bowel syndrome, aches, antiseptic, cyanogenetic, diuretic, dysentery, flu, prostatitis, snake bites, and spasms [34,35]. An *in vivo* study conducted by Bahekar and Kale [30] also suggested that *G. inodorum* was a reliable source of plant antioxidant.

O. sanctum Linn. is known as vitalizer and increases physical endurance. The *in vivo* hypoglycemic, hypolipidemic, and antioxidant properties of *O. sanctum* were evaluated and reported [36]. A full pharmacological importance of *O. sanctum* has been reviewed by Pattanayak et al. [19]. *O. sanctum* is used in the siddha medicine for the treatment of nephrotic disorders [37,38].

The current study also evidences the antioxidant properties of the selected plant samples. The data indicated that *T. belerica* Roxb. exhibited high TEAC ability and DPPH-based free radical scavenging capacity and *A. luzonensis* Gray. showed high superoxide scavenging activity when compared to that of the other tested samples (Figs. 2-4). The best 5 plants were categorized based on the GAE, TEAC, and antioxidant activity, which are assessed in this study, and are tabulated in Table 2.

CONCLUSION

The current study results conclude that *T. belerica* Roxb. was a potent source of antioxidant compounds compared to other tested samples. Moreover, the study estimates only the phenolic content of the plant sample. Other than phenolic acids, other phytochemicals are responsible for the free radical scavenging activity, which was demonstrated in the study. For example, *P. emblica* had high GAE score but reduced superoxide activity compared to the that of the other plant samples. Nevertheless, the extraction methods also influence the

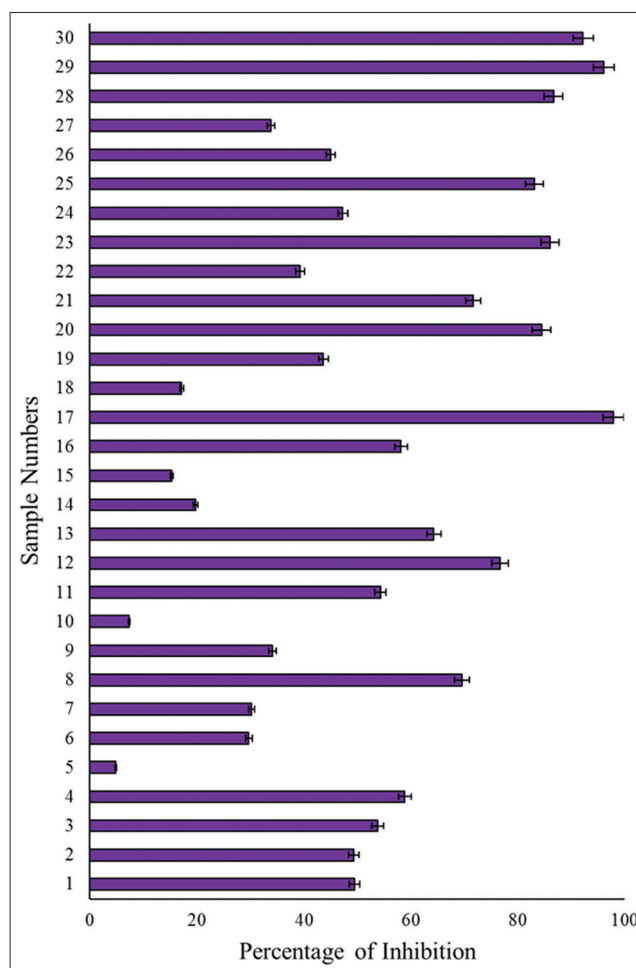


Fig. 4: Percentage of inhibition of superoxide radical by plant extracts

phytochemical content and the bioactivity. Thus, the further detailed study is needed to understand the complexity and distribution of bioactive compounds present in the commonly used plant species of Thailand that can be used to improve nutraceutical or cosmetic product with plant antioxidants.

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