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PHARMACOLOGICAL POTENTIALS OF MEDICAGO SATIVA L: A PROMISING OPTION FOR COVID-19 PREVENTION AND CURE

Nwaehiri Uloma L.[1], Akwukwaegbu Peter I.[2]* and Ibe Ijeoma J.[3]

[1]Department of Environmental Biology Federal Polytechnic Nekede Owerri.
[2]Department of Biochemistry, University of Port Harcourt Choba Rivers State.
[3]Department of Microbiology Federal Polytechnic Nekede Owerri.

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***Corresponding Author**
Akwukwaegbu Peter I.
Department of Biochemistry,
University of Port Harcourt
Choba Rivers State.

ABSTRACT

Corona Virus Disease 2019 (COVID-19) broke out in 2019 and spread rapidly around the world. According to World Health Organization (WHO) COVID 19 became a pandemic in March, 2020 and has affected more than 203 countries. Scientists are endeavoring to discover drugs for its efficacious treatment. Multiple drug trails are going world-wide. However; there are no vaccines and no specific antiviral therapy to the current pandemic. There is necessity for discovery of medicinal plant that will serve as a traditional contemporary means of treatment for COVID-19 while Scientists continue the search for a breakthrough. In this regards, *Medicago sativa L.* was assayed to determine the bioactive compounds that could potentially help in the treatment of covid-19 disease for the time being. The leaf, stem and root of *Medicago sativa L.* were screened separately for phytochemicals, secondary metabolites (*β*-sitosterol, stigmasterol, quercetin, glycyrrhizin kaempferol, licochalcone, liquiritigenin, glycerol, and 18β-glycerrhetinic acid) and therapeutic effect using standard analytical methods. Flavonoids, saponins and phenols were predominant in the plant extract while *β*-sitosterol, 18β-glycerrhetinic acid and glycyrrhizin were the common metabolites. ABTS, DPPH, superoxide and nitric oxide ion radical scavengers increased with increase in concentration while FRAP radical scavenger decreased with increase in concentration. The therapeutic potentials revealed that the plant extracts were mostly antipyretic, expectorant, cough-suppressing and dampness-resolving from traditional descriptions. In conclusion, the presence of these bioactive compounds signals immunoregulatory activities of *Medicago sativa* extract while

the therapeutic potential makes it ideal for formulation of herbal supplement against covid-19.

KEYWORDS: COVID-19, *Medicago sativa L.*, antioxidants, secondary metabolites, therapeutic effect.

1.0 INTRODUCTION

The coronavirus belongs to a family of viruses that may cause various symptoms such as pneumonia, fever, breathing difficulty, and lung infection.[1] These viruses are common in animals worldwide, but very few cases have been known to affect humans. The World Health Organization (WHO) used the term 2019 novel coronavirus to refer to a coronavirus that affected the lower respiratory tract of patients with pneumonia in Wuhan, China on 29 December 2019.[2, 3]

Although it is still too early to predict susceptible populations, early patterns have shown a trend similar to Severe Acute Respiratory Syndrome (SARS) and Middle East respiratory syndrome (MERS) coronaviruses. Susceptibility seems to be associated with age, biological sex, and other health conditions.[4] COVID-19 has now been declared as a Public Health Emergency of International Concern by the WHO.[3] Given the spread of the new coronavirus and its impacts on human health, the research community has responded rapidly to the new virus and many preliminary research articles have already been published about this epidemic.

The populations highly susceptible include elderly people, people with poor immune function, people with chronic co-morbidities, people with long-term use of immunosuppressive agents and persons with surgery history before admission.[5]

Rather than conventional Western medicine, the use of herbal medicine is mainly based on traditional knowledge and professional experience. *Medicago sativa L.* (Alfalfa) is popular herb that is commonly known as Lucerne. It belongs to the Leguminosae family; and referred as "father of all plants". In folk medicine, this herb is used in alternative herbal treatments. The medicinal value of the plants lies in their phytochemical components which produce definite physiological actions in the organism. It has high contents of tannins, pectin substances, saponines, amines, coumarin derivatives, triterpene glycosides, carotenoids, purines base, plant sterols, phytoestrogens (cumestrol), flavones, isoflavonoids and phenolic compounds.[6] In general, herbal formulations can be comprehensively analyzed from several modern approaches, particularly using computational analysis of traditional herbal knowledge with modern pharmacological perspectives.[7] This study was carried out to analyze the bioactive components in *Medicago sativa* and therapeutic potentials which supports it usage in the prevention and cure of covid-19 disease. The specific analysis carried out include: phytochemical screening, antioxidant, triterpenoids and secondary metabolites in the leaf, stem and root of *Medicago sativa*.

2.0 METHODS

2.1 Sample collection

Fresh leaf, stem and root samples of *Medicago sativa* were obtained from a local farm in "Uratta community", a village in Owerri North L.G.A, Imo State, Nigeria. Identification and authentication of leaf sample was done at the Plant Science Department of Federal Polytechnic Nekede, Owerri. Sample extraction and analysis was carried out according to the clinical guidelines of *Recommended Approaches for COVID-19 Diagnoses and Treatments*.[8] Phytochemical screening, antioxidant potentials, determination of triterpenoids and characterization of flavonoids secondary metabolites was analyzed. The total bioactive components obtained was categorized into groups according to their traditional therapeutic effects as; heat-clearing class, expectorants, cough-suppressing, exterior-relieving, blood activating, interior warming, resuscitation-inducing, tonic, regulating, astringent, antipyretic and hemostatic class.[7]

2.2 Extraction of the *M. sativa* leaf, Stem and Root

Leaf, stem and root collected were separated manually from aerial parts and washed with tap water prior to freeze–drying. Aliquots of 20 g from each dried and grounded plant part was extracted in 85 mL solvent consisting of methanol 90% in water acidulated by 1% hydrochloric acid (M), or acetic acid (AA), or distilled water (W). After 30 min sonication, centrifugation and filtration, the clear extracts were kept in the deep freezer until analysis.[9]

2.3 Analysis of parameters

2.3.1 Phytochemical screening

The samples were analyzed for their constituent phytochemicals using a Buck Scientific Gas Chromatography Analyzer as stipulated by Agomuo *et al*.[10]

2.3.2 Determination of in-vitro antioxidant properties

The method of Pellegrini *et al.*[11] was adopted for the determination of the ABTS radical inhibition potentials, the procedure of Shimada *et al.*[12] was applied for the determination of DPPH radical scavenging potentials, FRAP analysis was carried out according to the method of Sutharsingh *et al.*[13], superoxide radical scavenging assay was carried out using the method of Fontana *et al*. [14] while nitric oxide inhibition potentials was done according to the procedure of Alam *et al*.[15]

2.3.3 Determination of secondary Metabolites and Therapeutic effects

The secondary metabolites and therapeutic effects were obtained from a dataset of standardized names according to *Chinese Pharmacopoeia*[16] and *Chinese Materia Medica*.[17]

2.4 Method of data analysis

Data was obtained in triplicates and analyzed using Statistical package for Biological and Social Sciences (SPSS) Incident 21.0 Software. Descriptive and one way Analysis of Variance (ANOVA) for comparison was carried out and results expressed as mean ± standard error of mean (M±SD). Confident level of determination was ($p<0.05$).

3.0 RESULTS AND DISCUSSION

3.1 Phytochemical Screening of *M. sativa* leaf, Stem and Root

Table 1: Phytochemical content of *medicago sativa L.*

Parameters	Percentage (%)		
	Leaf	Stem	Root
Alkaloids	1.96±0.05	1.89±0.08	2.77±0.18
Saponins	5.05±0.88	6.23±0.92	6.56±0.99
Tannins	0.78±0.02	0.73±0.03	1.22±0.44
Flavonoids	7.66±0.51^{a}	6.13±0.45^{a}	10.87±0.66^{b}
Steroids	2.01±0.15	1.97±0.08	2.56±0.42
Phenols	3.24±0.22	4.110±0.16	5.05±0.40

Data are expressed as mean ± standard error of mean. Values bearing different superscript letter "a, b" across the row show significant difference ($P<0.05$).

3.2 Antioxidant potential

Figures 1-5 represented below showed the free radical scanvenging activity of ABTS, DPPH, nitric oxide, superoxide and FRAP respectively of the in-vitro antioxidants. The ABTs, DPPH, Super peroxide and nitric oxide increased with increase in concentration, while FRAP decreased with increase in concentration.

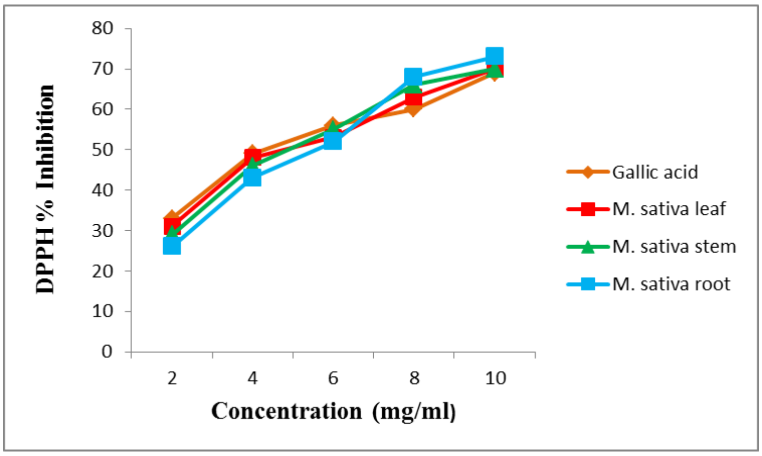

Figure 1: DPPH radical scavenging potentials of Gallic acid compared with the leaf, Stem and Root of *M. sativa L.*

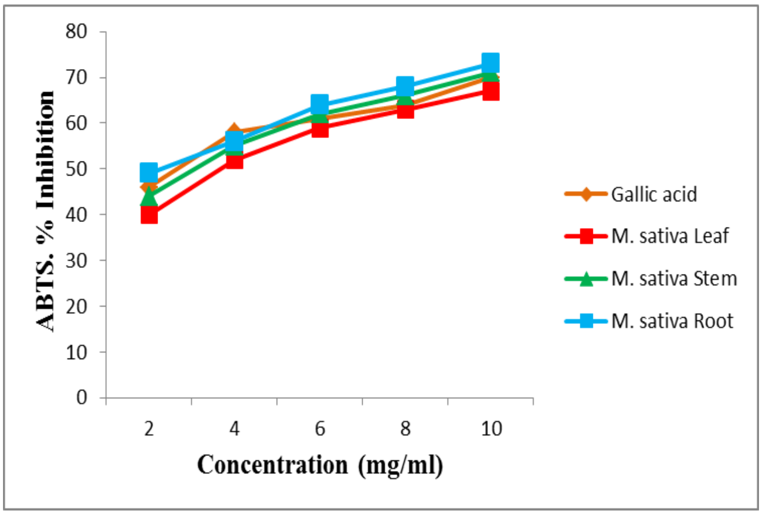

Figure 2: ABTS radical scavenging potentials of Gallic acid compared with the leaf, Stem and Root of *M. sativa L.*

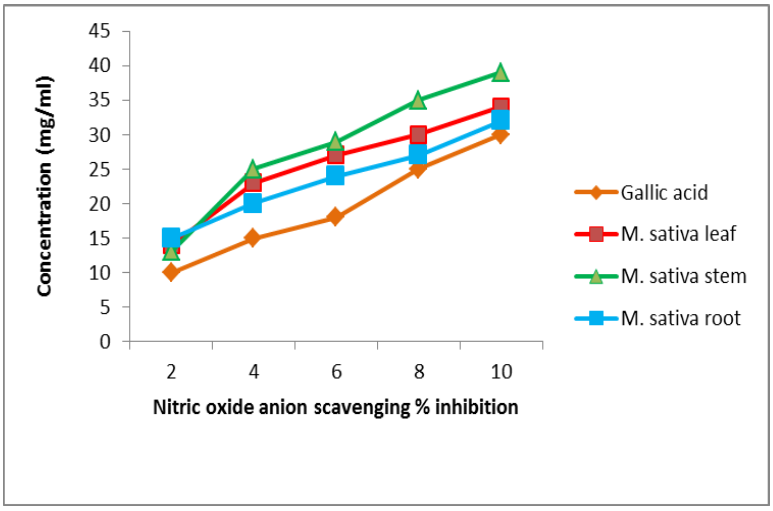

Figure 3: Nitric oxide radical scavenging potentials of Gallic acid compared with the leaf, Stem and Root of *M. sativa L.*

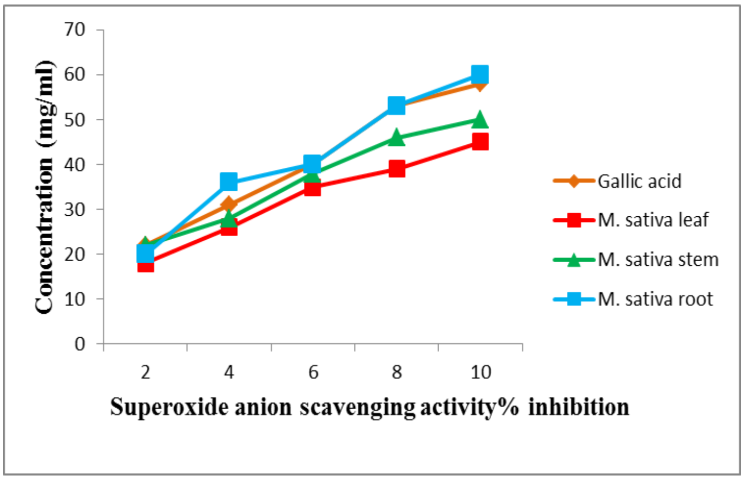

Figure 4: Superoxide radical scavenging potentials of Gallic acid compared with the leaf, Stem and Root of *M. sativa L.*

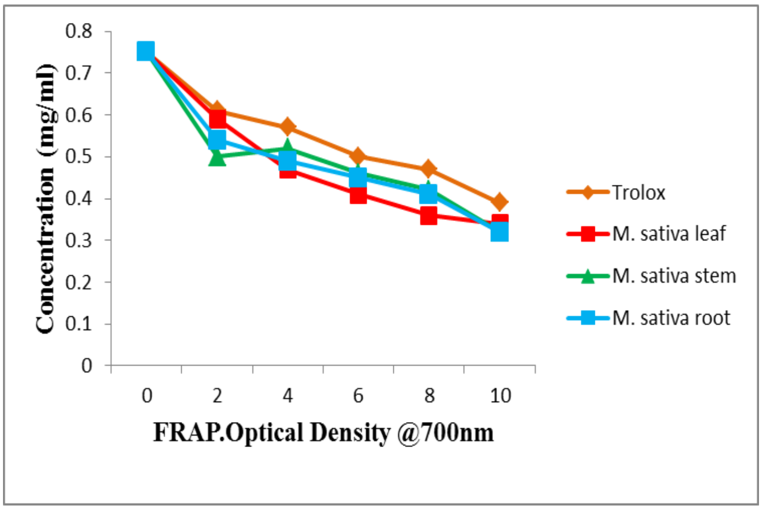

Figure 5: FRAP Optical Density radical scavenging potentials of Trolox compared with the leaf, Stem and Root of *M. sativa L.*

3.3 Secondary metabolites

Table 2: Determination of secondary metabolites.

The table below expressed the secondary metabolite present in leaf, stem and root of *M. sativa L*.

Secondary metabolite	Leaf	Stem	Root
β Sitosterol	+++	++	+++
Licochalcone	+	++	++
18β- glycerrhetinic acid	+++	++	++
Stigmasterol	+	+	++
Quercetin	++	++	++
Kaemferol	-	-	+
Glycyrrhizin	+++	++	+++
Liquiritigenin	++	+	+

Key: + low; ++ medium; +++ high; - (not detected)

3.4 Therapeutic effects

The figure below expressed the therapeutic effects of the plant extract

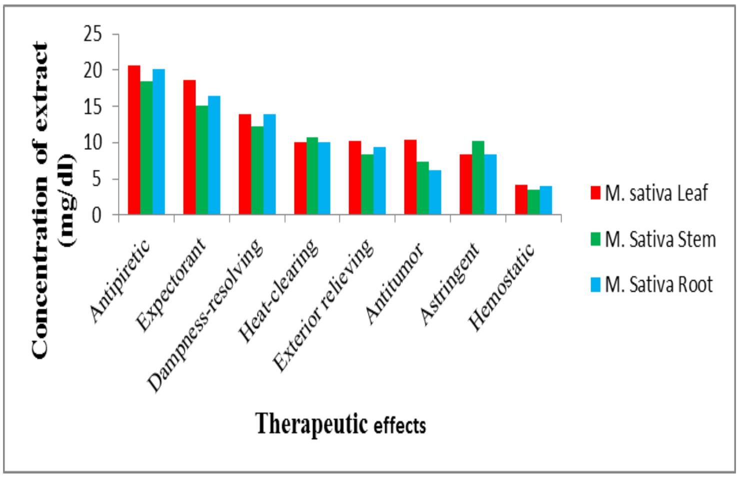

Figure 6: Therapeutic effects of *M. sativa L.* plant extract.

The therapeutic effects were expressed in a decreasing order from antipyretic to hemostatics.

4.0 DISCUSSION

The result of the phytochemicals composition of *Medicago sativa L.* leaf, stem and root extracts are shown in Table 1. The phytochemical assay revealed the presence of alkaloids, saponins, tannins, flavonoids, steroids and phenols. Tannins are protective substances that are astringent in nature and aids wound healing.[18,19] It also have some pharmacological activity like soothing relief, anti-inflamatory, regeneration of skin hemorrhoids, diuretics, frostbite and bum.[20] Saponins are stated to have hypercholestrolaemic, hyperglycaemic, hemolytic and anti-hypertensive effects.[19] It is a good expectorant and acts as cough suppressant.[21] Phenols are known to protect the cells against oxidative damage, decrease the risk of cancer formation and antioxidant potential.[22] The high content of phenol proves their antifungal, antimicrobial, antitumor, anti-inflammatory and antiseptic potentials.[22] Flavonoids are pharmacologically active and show great potential in the treatment of inflammation, cancer and virus-related diseases.[23] There was a significant difference ($P<0.05$) in the flavonoid content in leaf, stem and roots. Its anti-inflammatory and anti-oxidative effects are believed to be related with the inhibition of cytokine, nitric oxide (NO), chemokine, and growth factor production in macrophages.[24] The values of saponins, phenols and flavonoids are higher when compared to values obtained by Akwukwaegbu *et al.*[25] and Trease and Evans.[22]

Antioxidants exhibit their actions as free radical scanvengers against lipid peroxidation and other processes mediated by free radicals, and in the process shields the body from damage and diseases related to free radical reactions.[26] The free radical scavenging actions of these antioxidants are presented in Figures 1-5. The activities of these free radicals in the formulated diets were concentration dependent. ABTS, DPPH, superoxide and nitric oxide ion radical scavengers increased with increase in concentration while FRAP radical scavenger decreased with increase in concentration. The scavenging action might be attributed to the activities of phenol. Results from this study are similar to findings of Olangunju and Sandewa[27] and Madurangi and Gunathilake[28] who reported a high DPPH radical scavenging activity for dietary soursop; soursop pulp and beverage respectively.

Glycyrrhizin and 18*β*-glycyrrhetinic acid have anti-inflammatory ability as plant extract containing these secondary metabolites are believed to be potent against human immunodeficiency virus (HIV), SARS-CoV, herpes simplex virus (HSV), influenza virus (IAV-H3N2), rotavirus, enterovirus, coxsackievirus, varicella zoster virus, and respiratory syncytial virus.[29] Studies have shown that glycyrrhizin and 18*β*-glycyrrhetinic acid suppress proinflammatory cytokine cyclooxygenase-2 (COX-2), DPPH radicals and inhibit the translocation of toll-like receptors to lipid rafts.[30] *In vitro* studies have shown that glycyrrhizin inhibits HIV by preventing the virus from replication.[31] Additionally, glycyrrhizin, 18*β*-glycyrrhetinic acid, licochalcone and liquiritigenin also expressed immunoregulatory activity.[32]

Luo *et al.*[7] had earlier recommended the ten following plants {Glycyrrhizae Radix et Rhizome (Gan Cao); Scutellariae Radix (Huang Qin); Armeniacae Semen Amarum (Ku Xing Ren); Lonicerae Japonicae Flos (Jin Yin Hua); Forsythiae Fructus (Lian Qiao); Ephedrae Herba (Ma Huang); Poria (Fu Ling); Pogostemon Cablin (Guang Huo Xiang); Citri Reticulatae Pericarpium (Chen Pi); and Platycodonis Radix (Jie Geng)} as herbal supplements for the formulation of Traditional Chinese Medicine (TCM) for the treatment of covid-19 disease as a result of their secondary metabolite content and therapeutic potential. The aforementioned plants were recommended as the major ingredients for herbal formulation owing to presence of *β*-sitosterol, 18β- glycerrhetinic acid, glycyrrhizin, stigmasterol, quercetin, licochalcone A, liquiritigenin, luteolin and glycyrol which induced several therapeutic effects such as antipyretic, expectorant, cough-suppressing, dampness-resolving, heat-clearing, exterior relieving, hemostatics, astringent and ruscitation-inducing.

Table 2 revealed that *Medicago sativa L.* leaf, stem and root extracts are rich in *β*-sitosterol, 18β- glycerrhetinic acid, glycyrrhizin, licochalcone, stigmasterol and liquiritigenin chemical compound while Figure 6 expressed high antipyretic, expectorant, dampness-resolving, heating-clearing, exterior-relieving, astringent, antitumor and hemostatics therapeutic effects which supports its usage as potential supplement for herbal formulation against covid-19 disease. Similar results were obtained by Luo *et al.*[7], however; the most abundant chemical components possibly related to the antiviral signing pathway obtained in this study were *β*-sitosterol, 18β- glycerrhetinic acid and glycyrrhizin as against *β*-sitosterol, stigmasterol, and quercetin.

5.0 CONCLUSIONS

In conclusion, the presence of these bioactive compounds signals immunoregulatory activities of *Medicago sativa L.* extract and indicates its potential usage as a novel immunomodulatory drug. Furthermore, the therapeutic potential makes it ideal for formulation of herbal supplement against covid-19.

Further evidence-based studies are needed to prove the therapeutic effects *Medicago sativa L* in defeating covid-19 disease.

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