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PHYTOCHEMICAL SCREENING AND IN VITRO ANTIOXIDANT STUDY OF SIX PLANTS USED FOR THE TREATMENT OF HYPERTENSION IN TRADITIONAL MEDICINE

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ABSTRACT

The aim of this work is the enhancement of *Parkia biglobosa*, *Acacia nilotica*, *Cleome gynandra*, *Agelanthus dodoneifolius*, *Lannea microcarpa* and *Anogeissus leiocarpus*, six (6) medicinal plants used in the treatment of hypertension. This study focused on phytochemical screening and determination of antiradical activity (ARA) by thin layer chromatography (TLC) of hexanic, dichloromethane, ethyl acetate and methanol extracts obtained by solid-liquid depletion of plant powders. Phytochemical screening revealed the presence of several families of secondary metabolites. The hexanic and dichloromethane extracts are rich in sterols and terpenes while the ethyl acetate and methanolic extracts are rich in polyphenolic compounds such as flavonoids and tannins. Selective extracts showed an antiradical activity with respect to 2,2-diphényl-1-picrylhydrazyle (DPPH). Comparison of the results of the phytochemical and anti oxidant activity screening revealed the type of corresponding chemical compound for each revealed antiradical zone.

KEYWORDS: Hypertension, antioxidant activity, phenolic compound, flavonoids.

1. INTRODUCTION

Nature had been a source of medicinal plants since the beginning of life on the land; Interest in these medicinal plants has grown enormously thanks to the growing use of herbal products as natural cosmetics and self-medication by the general public for their biological effects. According to the WHO, more than 80% of the world's population depends on herbal medicines for their primary health care needs.[1,2]

Plants with their wide variety of chemical constituents offer promising source of medication for several diseases including hypertension. Anti hypertension properties of medicinal plants are being increasingly reported from different parts of the world. Plant based natural constituents can be derived from many part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc.[3] Biologically active compounds can be isolated from African medicinal plants by bioassay-guided fractionation procedures, in which various screening methods are employed to locate the desired activities in the crude extracts and in the fractions issuing from the different separation steps.[4] these plants contain biologically active chemical compounds such as alkaloids, terpenoids, saponins, phenolics, flavonoids etc. these phytocomposites or secondary metabolites have shown significant potential in the treatment of human diseases such as hypertension, oxidative stress.[3, 4-5] Hypertension is one of those diseases that is constantly growing. It is a major public health problem in developing countries. It affects 10 to 15% of the adult population in black Africa[6], with higher rates in urban areas.[7,8]

Although its exact causes are still little known, some authors associate it with oxidative stress.[9-14] Thus, several species are used in the treatment of various pathologies including hypertension. As a result, we were interested in *Parkia biglobosa, Acacia nilotica, Cleome gynandra, Agelanthus dodoneifolius, Lannea microcarpa and Anogeissus leiocarpus*, plants used in the traditional treatment of hypertension In Burkina Faso.[15-20] This work aims to contribute to the valorization of these medicinal plants through phytochemical screening and the determination of the antiradical activity (ARA) by thin layer chromatography (TLC).

2. MATERIALS AND METHODS

2.1. Plant material

The plant material consists of six (6) plants traditionally used in the treatment of hypertension. The information concerning the parts of the plant used are given in **Table 1**.

All the samples were collected, crushed and conserved in Department of traditional Medicine, Institute for health Science Research in Ouagadougou Burkina Faso (West Africa).

Table 1. Parts of the studied plants.

Plants	Parts
Parkia biglobosa (PB)	Trunk bark
Acacia nilotica var (AN)	Trunk bark
Cleome gynandra (CG)	Aerial part
Agelanthus dodoneifolius(AD)	Aerial part
Lannea microcarpa (LM)	Trunk bark
Anogeissus leiocarpa (AL)	Trunk bark

2.2. METHODS

2.2.1. Residual moisture content

The residual moisture content of the powders was determined according to the thermogravimetric method of the European Pharmacopoeia 6th edition in an oven (Memmert, Germany). The assay was performed in triplicate on one (01) gram of powder. The mean and standard deviation were calculated (n = 3, mean, standard deviation).

2.2.2. Preparation of extract

5 g of powder was successively extracted with n-hexane (50 mL x 3), dichloromethane (50 mL x 3), ethyl acetate (50 mL X 3) and methanol (50 mL x 3) by maceration during by stirring on a magnetic stirrer for 24h. The filtrated extracts were evaporated to dryness with a Rotavapor.

2.2. 3. Fingerprints of selective extracts

Phytochemical analysis of the fractions by thin layer chromatography (TLC) was carried out on a 60 F254 silica gel plate in glass, Merck (10 cm x 5 cm). selective extracts were spotted on the plate and developed in different eluent systems (**Table 2**).

Table 2. Solvent systems used for extraction and the different eluent for TLC.

Extracts	Eluent
hexane	Hexane-ethyl acetate -methanol (7-2-1 ; V/V/V)
dichloromethane	Toluene – Ethyl acetate - acetic acid (5-4-1 ; V/V/V)
Ethyl acetate	Ethyl acetate –methanol - Water (7-2-1 ; V/V/V)
methanol	Ethyl acetate –methanol - Water (7-2-1 ; V/V/V)

2. 2.4. Phytochemical screening

The phytochemical screening was performed on chromatoplates (60 F_{254}, 10 x 5 glass support, Fluka-Silica gel) following the methods described in the literature.[21-23] Each dry extract will be solubilized in its extraction solvent and 5µl are deposited on the TLC plate for the development of the chromatogram. The chromatography is developed over a path of 8 cm in the following solvent systems (**Table 2**): the main chemical groups sought by thin layer chromatography (TLC) are: Steroids, Terpenes and Phenolics. Several specific reagents served to reveal these groups of compounds: Sulfuric vanillin reagent for terpenes and sterols; Neu's reagent for flavonoids; $FeCl_3$ reagent for tannins and phenolic compounds.

2.2.5. Antiradical activity by TLC

The technique used to determine the antiradical activity of the extracts is based on the methodology developed by Takao with slight modifications.[24] DPPH (1,1-diphenyl-2-picrylhydrazyl), the reference oxidant, is dissolved in methanol at a concentration of 2 mg / mL and is used for spraying TLC plates after migration.

3. RESULTATS AND DISCUSSION

3.1.Residual moisture content

The residual moisture content of all plant powders is less than 10%. Thus, the various powdered plants can be stored for a long time without moulds or yeasts.[25-27] Indeed, the presence of water creates an environment unfavourable to conservation with the development of germs and undesirable reactions. These phenomena destroy the secondary methabolics, resulting in the ineffectiveness of the sample containing them. The residual moisture contents are expressed in the form of a histogram (**Figure 1**).

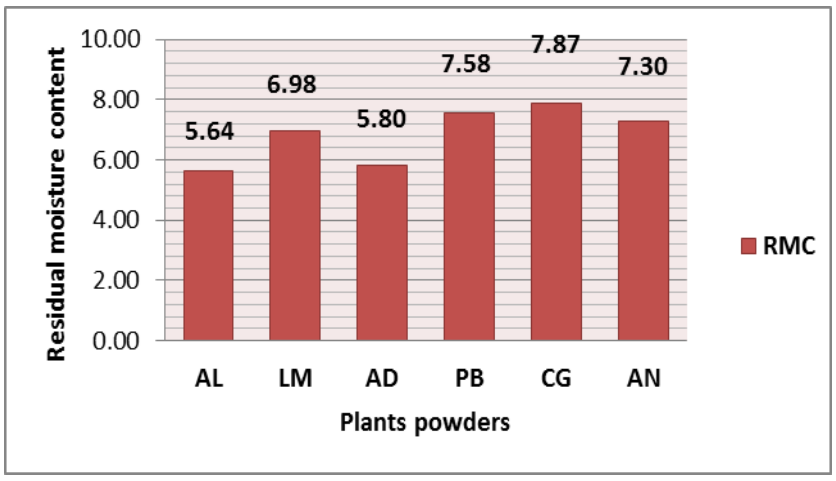


Figure 1: Resudual moisture content (RMC) expressed in percentage (%).

3.2. Extraction

Extractions with the various solvents led to dry extracts whose masses were determined by weighing. The yields of the extractions were subsequently determined by the following formula:

$$R\ (yield) = (m / M) * 100$$

m: mass of the extract obtained

M: mass of the plant material used

The results are expressed as a percentage (%) and recorded in **Table 3**. The best yield with hexane is obtained with the powder of *Agelanthus dodoneifolius*. It can be said that the powder of Agelanthus dodoneifolius will be rich in apolar compounds compared to other plants used in our study. With dichloromethane, the best yield is obtained with *Cleome gynandra powder*, which could therefore be the richest plant in medium polar compounds. As for the extraction with the polar solvents namely ethyl acetate and methanol, the best extraction yield is obtained with the *Acacia nilotica* powder. This would mean that the bark powder of *Acacia nilotica* would be rich in polar compounds such as flavonoids, tannins and other phenolic compounds.

Table 2: Extracts and yields.

	Hexane	***Dichloromethane***	***ethyle Acetate***	***Methanol***
Parkia biglobosa	0,325	0,25	0,405	13,55
Acacia nilotica	1,075	0,2	6,23	30,4
Cleome gynandra	0,65	1,28	1,09	8,14
Agelanthus dodoneifolius	2,79	0,85	0,63	18,85
Lannea microcarpa	0,42	0,07	0,39	16,83
Anogeissus leiocarpus	0,33	0,135	0,39	15,42

3.3. Fingerprints of selective extracts

Fingerprints have been developed for all selective extracts. They make it possible to highlight all the components of each plant studied. The monographs of European and African traditional medicine identify plants through macroscopic and microscopic characteristics as well as thin layer chromatography. In addition to identification, it is also possible to carry out quality control or standardization using fingerprints to ensure that the recipe is reproducible.The fingerprints of hexanique extracts by thin-layer chromatography of plants extracts are shown in **figure 2**.

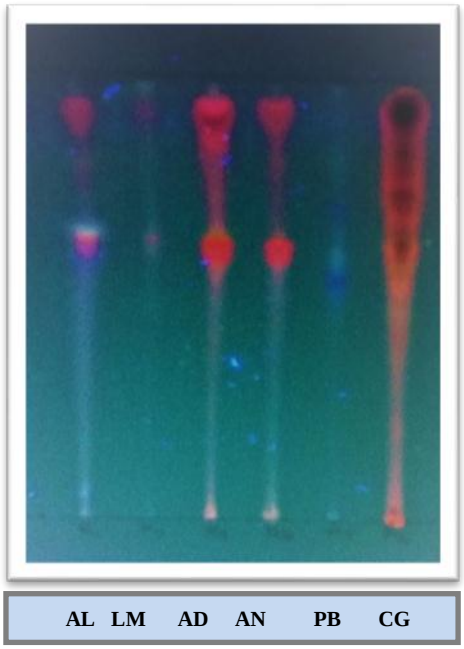


Figure 2: Fingerprint of hexanic extracts by TLC Solvant system: Hexane- ethyle acetate-methanol Observation : under ultraviolet (366 nm).

3.4. Phytochemical screening

Phytochemical screening was performed on the extracts obtained by successive exhaustion with different solvents with increasing polarity. The different chromatographic profiles (**Figures 3-5**) show that the hexanic and dichloromethane extracts are rich in sterols and terpenes; ethylacetatics and methanolic flavonoids, tannins and phenolic compounds.

The spots with the different colorations observed on the chromatograms can correspond to several classes of secondary metabolites.

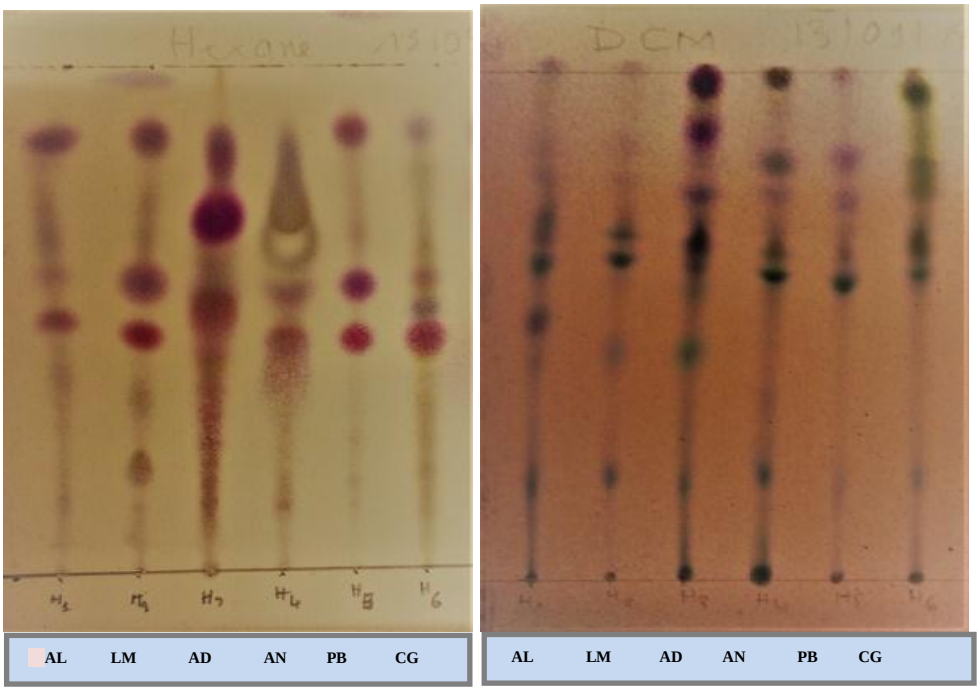
The colours purple and blue on the chromatograms of **the figure 3** observed in daylight after spray with sulfuric vanillin and heating at 105 ° C during observation, characterize sterols and terpenes. The green, blue, yellow-orange coloring observed under UV at 366 nm wavelength after Neu reagent spraying characterizes the presence of flavonoids in our extracts (**figures 4**). Secondary metabolites have various biological properties including antihypertensive properties. The actual presence of some of them in our extracts would partly explain the therapeutic properties of the plants studied. Indeed, flavonoids are antioxidants known for excellence.[25-29]

In addition to their antioxidant power, they are anti-ulcer, antitumor, antispasmodic, anti-secretory and antidiarrheal[24], anti-allergic, anti-inflammatory, hypotensive and protect

against cancer and cataracts.[25] They are also endowed with aphrodisiac virtues.[26] Tannins have an astringent effect and exhibit vitamin P properties. They strengthen blood vessels and contribute to the accumulation of vitamin C in the body. Polyphenols have a potassium retention effect and therefore exhibit diuretic activity.[27] Sterols and terpenes have bactericidal properties.[27] In our study, we were able to identify in the extracts the main chemical compounds likely to be at the origin of antihypertensive activities. Next, we want to highlight the correlation between this activity and antioxidant power.

Table 3. Phytochemical screening (+ *: present ; - : absent*).

Extracts	Regeants	Compounds	Samples	Results
Hexane	Sulphiric vanillin	Sterols and triterpenes	1- AL 2- LM 3- AD 4- AN 5- PB 6- CG	+ + + + + +
Dichloromethane	Sulphiric vanillin	Sterols and triterpenes	1- AL 2- LM 3- AD 4- AN 5- PB 6- CG	+ + + + + +
Ethyle acetate	Neu	Flavonoids	1- AL 2- LM 3- AD 4- AN 5- PB 6- CG	+ + + + + +
	$FeCl_3$	Phenolic compounds	1- AL 2- LM 3- AD 4- AN 5- PB 6- CG	+ + + + + +
Methanol	Neu	Flavonoids	1- AL 2- LM 3- AD 4- AN 5- PB 6- CG	+ + + + + +
	$FeCl_3$	Phenolic compounds	1- AL 2- LM 3- AD 4- AN 5- PB 6- CG	+ + + + + +



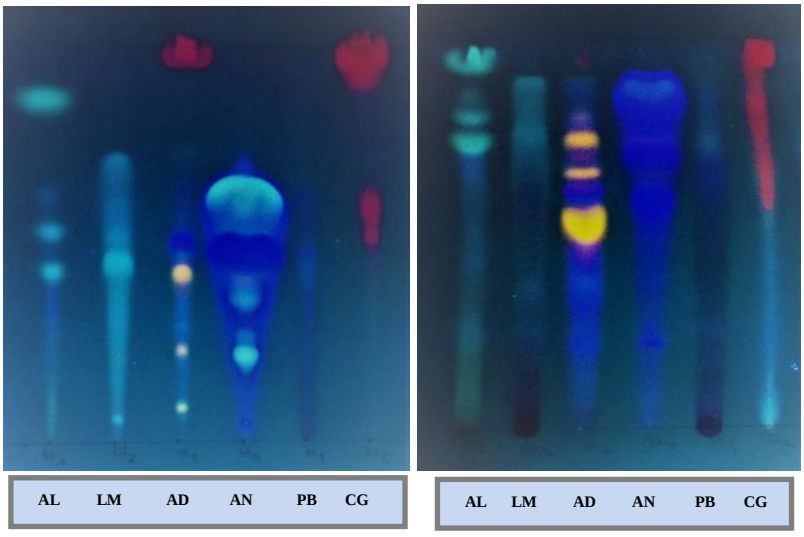
n-hexanic extracts **dichloromethane extracts**

Figure 3. Chromatographic profiles of n-hexanic and dichloromethane extracts.

Desired compounds: sterols and terpenes

Revelator: sulfuric vanillin reagent

Observation : light of day



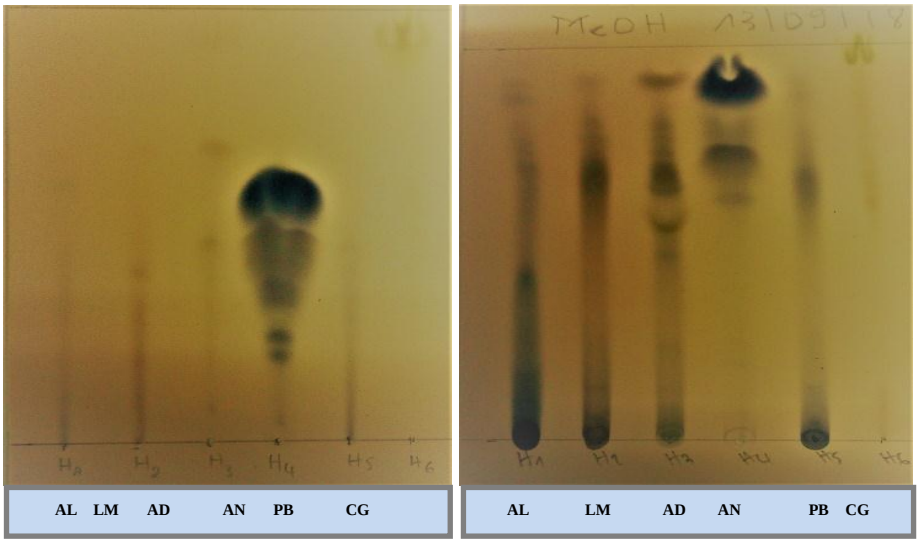
ethyl acetate extracts **methanolic extracts**

Figure 4. Chromatographic profiles of ethyl acetate and methanolic extracts.

Desired compounds: flavonoids

Revelator: Neu reagent

observation : under ultraviolet (366 nm)



ethyl acetate extracts **methanolic extracts**

Figure 5. Chromatographic profiles of ethyl acetate and methanolic extracts.

Desired compounds: tannins and phenolic compounds

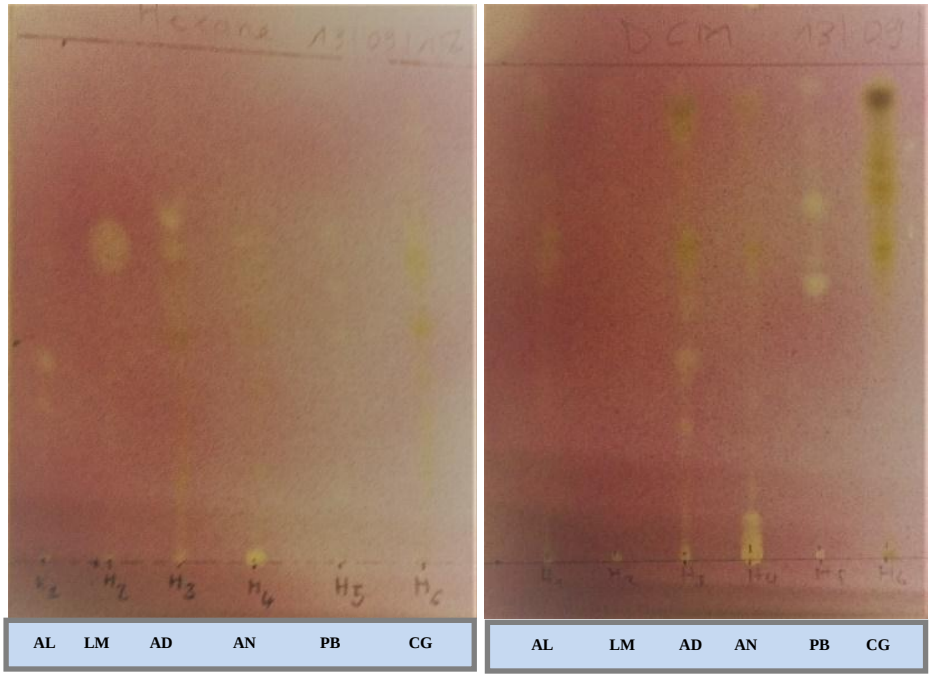
Revelator: 2% $FeCl_3$ in methanol

Observation : light of day

3.5. Determination of antiradical activity (ARA)

The figures below show the presence of light yellow spots on a purple background. This shows that some extracts contain phytocomposites that can trap free radicals.[18, 30]

By superimposing the chromatographic profiles of phytochemical screening (**Figure 3-5**) and those of antioxidant activity (**Figure 6-7**), the correspondence between the active zones and the phytocompounds responsible for this activity was established. Indeed, the antiradical activity is more pronounced in ethyl and methanol acetate extracts, compared to hexane and dichloromethane extracts; this could be explained by the richness in flavonoids, tannins and phenolic compounds of acetatic and methanolic extracts. Antiradical activity may therefore explain the use of these plants in the traditional treatment of hypertension.

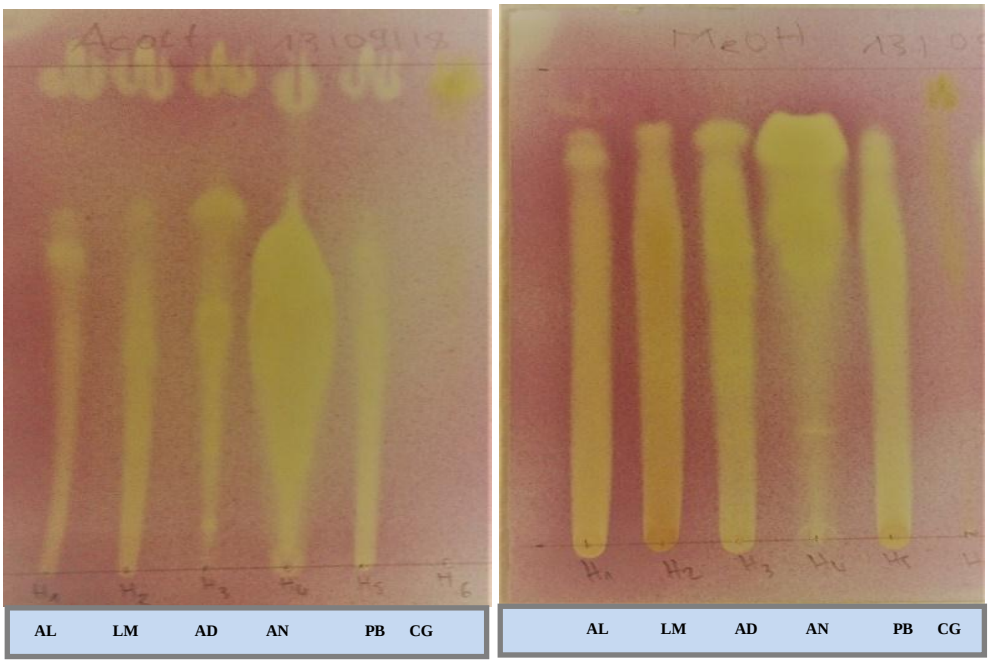


n-hexanic extracts **dichloromethane extracts**

Figure 6. Chromatographic profiles of radical activity on TLC extracts.

Revelator: DPPH reagent

Observation: light of day



ethyl acetate extracts **methanolic extracts**

Figure 7 : Chromatographic profiles of radical activity on TLC extracts.

Revelator: DPPH reagent

Observation: light of day

4. CONCLUSION

This study allowed us to highlight the presence of secondary metabolites such as steroidal compounds, terpenic compounds and phenolic compounds in various extracts. Most of these secondary metabolites have remarkable antioxidant and biological activities. *Anogeissus leiocarpus*, one of the plants that has been studied in depth, has shown a high presence of phenolic compounds, which is an indicator of anti-free radical activities. For this plant, work on the determination of the main chemical groups and the quantification of antioxidant activity by spectrophotometry and the isolation of tracer molecules will be the subject of further studies.

REFERENCES

1. Shanmugasundaram S. Complementary and Alternative therapies in palliative care; a transition from modern medicine to traditional medicine in India. Journal of Cancer Pain & Symptom Palliation, 2005; 1(4): 25-29.
2. Agaie BM, Onyeyili PA, Muhammad BY, Landan MJ. Some Toxic Effects of Aqueous Leaf Extract of *Anogeissus leiocarpus* in rats. Journal of Pharmacology and Toxicology, 2007; 2(4): 396-401.
3. Okpekon T, Yolou S, Gleye C, Roblot F, Loiseau P, Bories C and al. Antiparasitic activities of medicinal plants used in Ivory Coast. Journal of Ethnopharmacology, 2004; 90(1): 91-97.
4. Vonthron-Sénécheau C, Weniger B, Ouattara M, Bi FT, Kamenan A, Lobstein A and al. In vitro antiplasmodial activity and cytotoxicity of ethnobotanically selected Ivorian plants. Journal of Ethnopharmacology, 2003; 87(2-3): 221-225.
5. Adejumobi JA, Ogundiya MO, Kolapo A, kunade MB. Phytochemical composition and in vitro antimicrobial activity of *Anogeissus leiocarpus* on some common oral pathogens. Journal of Medicinal Plants Research, 2008; 2(8): 193-196.
6. Olatunbosun ST, Kaufman SJ, Cooper R, Bella AF. Hypertension in a black population: prevalence and biosocial determinants of high blood pressure in a group of urban Nigerians. Journal of Human Hypertension, 2000; 14: 249-257.
7. Cooper R, Rotimi C, Ataman S, McGee D, Osotimehin B, Kadiri S and al. The prevalence of hypertension in seven populations of West African origin. American Journal of Public Health, 1997; 87(2): 160-168.

8. Van Rooyen JM, Kruger HS, Huisman HW, Wissing MP, Margetts BM, Venter CS et al. An epidemiological study of hypertension and its determinants in a population in transition: the THUSA study. Journal of Human Hypertension, 2000; 14(12): 779-87.
9. John S & Schmieder RE. Impaired endothelial function in arterial hypertension and hypercholesterolemia: potential mechanisms and differences. Journal of Hypertension, 2000; 18: 363-374.
10. Yasunari K, Maeda K, Nakamura M & Yoshikawa J. Oxidative stress in leukocytes is a possible link between blood pressure, blood glucose, and C-reacting protein. Hypertension, 2002; 39: 777-780.
11. Madamanchi NR, Vendrov A & Runge MS. Oxidative stress and vascular disease. Arteriosclerosis, Thrombosis and Vascular Biology, 2005; 25(1): 29-38.
12. Heistad DD. Oxidative Stress and Vascular Disease: 2005 Duff Lecture. Arteriosclerosis, Thrombosis and Vascular Biology, 2006; 26(4): 689-695.
13. Violi F, Cangemi R, Brunelli A, Madamanchi N R & Runge M. Oxidative Stress, Antioxidants and Cardiovascular Disease. Arteriosclerosis, Thrombosis and Vascular Biology, 2005; 25(1): 29-38.
14. Madamanchi NR, Tchivilev I & Runge M. Genetic markers of oxidative stress and coronary atherosclerosis. Current Atherosclerosis Reports, 2006; 3(8): 177-183.
15. Ouédraogo S, Sombié BC, Ouédraogo JCW, Traoré TK, Nitiéma M, Belmnaba L, Ouédraogo S, Semdé R, Guissou IP. Standardization of Extracts from trunks's Barks of *Lannea microcarpa* engl. and K. Krause (Anacardiaceae) and *anogeissus Leiocarpus* (DC) Guill. and Perr. (Combretaceae) for the Formulation of Antihypertensive herbal medicines. International Journal of Pharmaceutical Sciences Review and Research, 2018; 48(1): 92-97.
16. Traoré S, Ouédraogo S, Yoda J, Traoré TK, Traoré A, Lompo M, Kini BF, Ouédraogo S and Semdé R. Evaluation of *Parkia biglobosa* (Jacq.) trunk's bark extracts syrup formulation, The Pharma Innovation Journal, 2018; 7(11): 197-201.
17. Ouédraogo S, Traoré A, Somé N, Lompo M, Guissou IP, Schott C, Bucher B, Andriantsithohaina R. Cardiovascular properties of *Tapinanthus dodoneifolius* (DC. Danser) African Journal. Traditional, Complementary and Alternative Medicine, 2005; 2(1): 25-30.
18. Boussim IJ, Sallé G, Guinko S. Tapinanthus parasite du karité au Burkina Faso. 1ère partie: Identification et distribution. Bois et Forets des Tropiques, 1993; 238: 45-52.

19. Meda NTR, Bangou MJ, Bakasso S, Millogo-Rasolodimby J, & Nacoulma O G. Antioxidant activity of phenolic and flavonoid fractions of *Cleome gynandra* and *Maerua angolensis* of Burkina Faso. Journal of Applied Pharmaceutical Science, 2013; 3(2): 36–42.
20. Sereme A, millogo-rasolodimby J, Guinko S, Nacro M. Proprietes therapeutiques des plantes a tanins du Burkina Faso, pharmacopée et médecine traditionnelle africaines, 2008; 15: 41–49.
21. Ladigina EY, Safronich LN, Otriacheva VE, Balandina IA, Grinkevich NI, Sorokina AA, Glizin VI, Molodojnikova LM, Mitin YS, Samilina IA & Ermakova VA. Analyse chimique des plantes médicinales, édition Moskva vischaya chkola (1983) 172 (traduit du russe).
22. Békro YA, Békro MJA, Boua BB, Trabi FH & Ehile EE. Etude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (Caesalpiniaceae). Sciences et Nature, 2007; 4: 217-225.
23. Lhuilier A. Contribution à l'étude phytochimique de quatre plantes malgaches :Agauria salicifolia Hook.f ex Oliver, Agauria polyphylla Baker (Ericaceae), Tambourissa trichophylla Baker (Monimiaceae) et Embelia concinna Baker (Myrsinaceae). Thèse de Faculté des Sciences Pharmaceutiques - Université Paul Sabatier - Toulouse III, 2007; 214.
24. Takao T, Kitatami F, Watanabe N, Yagi A & Sakata K. A simple screening method for antioxydants and isolation of several antioxydants produced by marine bacteria from fish and shell fish. Bioscience, Biotechnology and Biochemistry, 1994; 58: 1780-1783.
25. Europe C.D., Pharmacopée européenne 6ème Édition, ed. D.E.d.l.Q.d.M. & and S.d.S. (EDQM). 2007: Strasbourg, France. 1218.
26. Aiache J, Aiache S, and Renoux R, Initiation à la connaissance du médicament.-4 ème éd. 2001, Paris: Masson.-338.
27. Dawoodbhai S and Rhodes CT. The effect of moisture on powder flow and on compaction and physical stability of tablets. Drug Development and Industrial Pharmacy (1989); 15(10): 1577-1600.
28. Torel J, Cillard J & Cillard P. Antioxidants activities of flavonoids and reactivity with peroxy radical; Phytochemistry, 1986; 25: 383-385.
29. Husain SR, Cillard J & Cillard P. Hydroxyl radical scavenging activity of flavonoids; Phytochemistry, 1987; 26: 2489-2492.

30. Shahidi F, Wanasundara PK. Phenolic antioxidants; Critical Review in Food Scienceand Nutrition, 1992; 32: 67-103.
31. Harborne JB, Williams CA. Advances in flavonoid research since 1992; Phytochemistry, 2000; 55(6): 481-504.
32. D'abrosca D, Pacifico S, Cefarelli G, Mastellone C, Fiorentino A; 'Limoncella' apple, an Italian apple cultivar: Phenolic and flavonid contents and antioxidant activity; Food Chemistry, 2007; 104: 1333-1337.
33. Hugues A, N'guessan D, Camille ED, Mamyrbékova-Békro J A, Békro Y A. CCM d'extraits sélectifs de 10 plantes utilisées dans le traitement traditionnel de l'hypertension artérielle en Côte d'Ivoire. European Journal of Scientific Research, 2011; 66(4): 575-585.