Screening of medicinal plants from the Cerrado[(1)]

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RESUMO: Esse trabalho descreve uma breve triagem de plantas medicinais utilizadas na região do Triângulo Mineiro – MG. As plantas foram com extraídas com etanol, obtendo-se assim os extratos brutos. Os extratos brutos de cada planta foram avaliados através de Cromatografia em Camada Delgada (CCD), utilizando-se vários sistemas eluentes e como reveladores: Lâmpada UV (254 e 365 nm), Ácido Fosfomolíbdico, Ninidrina e Reagente de Dragendorff. Além disso, também foi realizada análise por Ressonância Magnética Nuclear de hidrogênio (RMN-^{1}H). Por fim, bioensaios de atividade antibacteriana e antifúngica foram realizados com cada extrato bruto. A partir dos resultados obtidos foi possível conhecer as classes de compostos presentes nos extratos brutos, além de identificar os extratos bioativos.

Termos de indexação: triagem, plantas medicinais, atividade biológica.

ABSTRACT*: This work describes a brief screening of medicinal plants found in the Triangulo Mineiro (Minas Gerais, Brazil) region. The plants were extracted using ethanol to produce the untreated extract. The untreated extracts from each plant were analysed through Thin Layer Chromatography (TLC), using various solvent systems and visualisation methods, such as UV Lamp (254 and 365 nm), Phosphomolybdic acid, Ninhydrin and Dragendorff reagent. Additionally, the samples were analysed using Proton Nuclear Magnetic Resonance (NMR-^{1}H). Finally, bioassays of antibacterial and antifungal activity were also performed with each of the untreated samples. With the results, it was possible to determine the classes of the compounds in the extracts, and also identify the existence of bioactive extracts.*

Index terms*: screening, medicinal plants, biological activity*

INTRODUCTION

In recent decades, there has been an increasing interest in alternative therapies and in the therapeutic use of natural products, especially those derived from plants. There are many reasons that explain this growing interest, such as the inefficacy of conventional medicine, the incorrect usage of synthetic drugs, the adverse effects of drugs currently in use, amongst others. However, the responsible authorities, due to the efficacy and safety of the fabrication processes, do not permit the usage of many of these substances. As a result, systematic research with medicinal plants, traditionally used by local people, represents an attractive approach for the discovery and development of new drugs. This research field has led to an increase in publications in this area, which has led private and government institutions to finance research programs all over the world (RATES, 2001). However, the potential of superior plants as a source of new drugs is still little explored (RATES, 2001).

The research and development of therapeutic materials from plants is a complex process that involves a multidisciplinarity approach due to the many basic sciences involved, such as biology, chemistry and pharmacology, not to mention biotechnology, which is necessary for large-scale industrial production of a new drug (RATES, 2001). The process of drug discovery from medicinal plants begins with a botanist, ethnobotanist, ethnopharmacologist or ecologist that collects and identifies the plant(s) of interest (BALUNAS *et al*., 2005). The collection can involve many species with known biological activity (e.g. commonly used medicinal plants) or it can be random for a big screening program. The phytochemists prepare the untreated extracts from the plants, submit these extracts to biological evaluation in relevant

pharmacological assays. With that, the isolation, purification and identification process of the active compounds begins, using bioassay guided fractioning (BALUNAS *et al*., 2005).

With the increase in resistant bacteria, and life threatening viruses, the reoccurring problems in transplanted patients, the huge increase in the incidence of fungi infections and cancer, which is responsible for 12% of the deaths around the world and is the second major cause of death in the west, it is necessary to find new compounds with chemical and biological potential (STROBEL *et al*., 2004). A lot has been done to find new bioactive compounds and marine organisms, plants and animals have been found to be rich sources of natural products with diverse biological activity (NEWMAN *et al*., 2007; BUTLER, 2004). It is estimated that there are between 250,000-300,000 species of superior plants in the world, from these, about 10,000 species have been reported linked with some medical use. This represents a minimum amount out of the approximate total, which shows that there is a great potential in research with plants with the aim of discovering new compounds with pharmacologic applications (McCHESNEY *et al*., 2007).

The discovery of penicillin by Fleming in 1928, the re-isolation and the clinical studies by Chain, Florey and collaborators in the 40's and the commercialization of synthetic penicillin revolutionised research with new drugs (ALDER, 1970; LAX, 2004; WAINWRIGHT, 1990; MANN, 1999; BUTLER, 2004). Paclitaxel, commercial name Taxol, is the best example of a natural product isolated from plants. This compound can be obtained from the bark extract of *Taxus brevifolia*. Taxol presents anticancer activity for breast and ovary cancers. Artemisinin, another important example of a natural product isolated from plants, was obtained from the Chinese plant *Artemisia annua* (PHILLIPSON, 2001).

According to Newman and Cragg between 1981-2006 1,184 new compounds were found that present the most varied biological activities, such as analgesic, anti-Alzheimer, anti-Parkinson, antibacterial, anticancer, anti-diabetes, anti-glaucoma, antiviral, immune suppressor, amongst many others (NEWMAN et al., 2007). From these, 111 are antibacterial agents currently in clinical usage, 77 are antiviral agents, 14 are anti-parasitic, and 32 are anti-diabetes. Also, according to other authors between 1940-2006, 252 anticancer drugs were introduced into the market, and 100 of them were discovered between 1981-2006 (NEWMAN et al., 2007).

Tea is the second most consumed liquid in the world, second only to water, and the two main types of tea are green and black (HODGSON, 2010). There are studies that suggest that regular tea consumption may reduce the risk of cardiovascular disease due to the large amount of flavonoids (KURIYAMA, 2008), which inhibit the development of atherosclerosis in animal models (DEBETTE et a., 2008; LOKE et al., 2008). There is limited evidence that suggests the benefits of green tea on weight loss and body fat (HODGSON, 2010). The results of studies in animal models suggest that regular intake of tea can lower blood pressure (HODGSON et al., 2006). Over 50 studies confirm the in vitro antioxidant activity of flavonoids (HALLIWELL et al., 2005; MANACH et al., 2005). Results of in vitro studies suggest that flavonoids in tea and other foods have an effect on inflammatory, consistent with anti-inflammatory mediators effect (SIES et al., 2005). Lambert and Elias (2010), performed studies in animal models of carcinogenesis and this studies shown that green tea can inhibit tumorigenesis during the initiation, promotion and progression stages. According to the authors, Green tea is rich in catechins, but although the catechins are chemical antioxidants, there is evidence that some of the effects of these compounds may be related to induction of oxidative stress. Pro-oxidant effects appear to be responsible for the induction of apoptosis in tumor cells and may also induce endogenous antioxidant systems in normal tissues that offer protection against carcinogenic insult (LAMBERT & ELIAS, 2010).

The *Equisetum arvense* tea is used in folk medicine as a diuretic, anti-inflammatory, astringent, among other applications [http://pt.wikipedia.org/wiki/Cavalinha_(planta) accessed on 2014, May 02]. There are few scientific studies on the biological activity of extracts of *Equisetum arvense*. However, Gürbüz and coworkers, studying the aqueous and methanol extracts of *Equisetum telmateia* and demonstrated that oral administration of these extracts showed significant stomach protection (77.9 % and 100 % inhibition of ulcers, respectively), against ulcer formation applied models (GÜRBÜZ et al., 2008). Gürbüz et.al (2008) studied the methanol extract of *Equisetum palustre* and showed that the extract of this specie also has stomach protection models applied to ulcer formation. Bhattarai et al (2009), conducted a screening of 40 medicinal plants used by the population of Nepal to treat bacterial infections. From this study, the authors found that the methanol extract of *Equisetum debile* showed antibacterial activity against *Bacillus subtilis* and *Pseudomonas aeruginosa*.

Mikania glomerata is a type of medicinal plant used popularly against flu, throat infection, cough and bronchitis. Despite showing many popular applications, there are few scientific studies on the action of *Mikania glomerata*. Among the studies it was observed anti-inflammatory activity *in vitro* and inhibitory activity of intestinal and uterine muscles *in vivo* (SALGADO et al., 2005). Furthermore, studies show high bronchodilator and antiallergic activity of the ethanol extract of Mikania glomerata (MOURA et

al., 2002; FIERRO et al., 1999). Freitas et al studied the effect of *Mikania glomerata* and *Mikania laevigata*'s extracts in the preventive treatment of pneumoconiosis, which is characterized by inflammation of the lungs due to exposure to coal dust (FREITAS et al., 2008). From this study the authors suggest that *M. glomerata* and *M. laevigata* may be good candidates for the prevention of oxidative lung injury caused by exposure to coal dust (FREITAS et al., 2008). Do Amaral et al. evaluated the antibacterial activity, against *Staphylococcus aureus* and PI57 MAOI, of the extracts of different polarities of *Mikania glomerata* (DO AMARAL et al., 2003). It was found from this study that only the hexane extract showed antibacterial activity. Furthermore, it was found that hexane and dichloromethane extracts were active against MAO - B, showing no activity inhibiting MAO-A, whereas the methanol extract inhibited MAO-A and MAO-B without selectivity (DO AMARAL et al., 2003).

The *Ginkgo biloba* extract is one of the most popular in Europe and is used to relieve symptoms associated with cognitive disorders (DEFEUDIS, 1991). Recently, *Ginkgo biloba* has been approved in Germany for the treatment of dementia. Its mechanism of action in the central nervous system is not yet fully known, but the main effect is apparently due to the synergistic antioxidant properties of flavonoids, terpenoids, and organic acids that constitute the extract of this plant. They act in the "capture" of free radicals which are considered mediators of excessive lipid peroxidation and cell damage observed in Alzheimer's disease (DEFEUDIS, 1991; PACKER et al., 1995; OYAMA & KATAYAMA, 1994; OKEN et al., 1998). The study by Watanabe and coworkers (WATANABE et al., 2001) showed that diets supplemented with *Ginkgo biloba* extract have a considerable *in vivo* neuromodulator effect. Furthermore, the study of Walesiuk & Braszko (2009) demonstrated that *Ginkgo biloba* extract prevents stress and memory.

The main purpose of this work was to perform a chemical and pharmacological screening with some medicinal plants used by the population of the Triangulo Mineiro region, in Minas Gerais, Brazil. To do so, extracts from *Equisetum arvense, Mikania glomerata*, *Ginkgo biloba* and 3 commercially available brands of green tea were tested and their chromatographic profile analysed, to find out the chemical nature of the compounds present in these plants. The crude extracts were also analysed by Proton Nuclear Magnetic Resonance (RMN-^{1}H) and antimicrobial activity bioassays.

MATERIAL AND METHODS

For this work the plants used were: *Equisetum arvense in natura* and dehydrated 3 different brands of green tea; dehydrated *Mikania glomerata* and dehydrated *Ginkgo biloba* fromt commercial establishments from Uberaba, MG. To obtain the extracts, 20 g of each plant were extracted, macerating the plants separately with 100 mL of ethanol. The plant/ethanol mixture was kept at rest for 15 hours. Subsequently, the mixture was filtered and the residue was re-extracted with 20 mL of ethanol, following the same protocol for 3 hours. Both filtered materials were gathered together and evaporated using a rotary evaporator to obtain the dry extracts. The extracts were named *Equisetum arvense in natura* (Eain) and dehydrated (Extract Ead), Green Tea (Gt.1, Gt.2 and Gt.3), Ginkgo biloba (Gb) e *Mikania glomerata* (Mg).

The extracts were analysed using Thin Layer Chromatography (TLC) using a mixture of diclorometha ne/ethanol (1:1) as eluent, and the following visualization methods: UV-vis Lamp (254 e 365 nm), Phophomolybdic acid, Ninhydrin and Dragendorff reagent. The extracts were also analysed by NMR-^{1}H, to do so, a little amount of each extract was dissolved in deuterated methanol and analysed (Bruker, model AVANCE III, 9,4 Tesla – 400 MHz for the frequency of hydrogen).

The extracts were also tested in biological assays for the evaluation of the antimicrobial activity using *E. coli* (Gram+), *S. aureus* (Gram-), *A. flavius* and *C. albicans* according to Bauer & Kirby (BAUER & KIRBY, 1966). For that, 250 mg/mL of each extract were diluted in ethanol and the filter paper discs of 7 mm were impregnated with extract solution. The discs were allowed to remain at room temperature until complete diluent evaporation and kept under refrigeration until ready to be used. The discs loaded with the extracts were placed onto the surface of the agar that was previous inoculated with a bacterial concentration of approximately 1-2x10^{8} UFC/mL. The diameter of inhibition halo of bacterial growth around each disk was measured in millimeters. These are related to the sensitivity of the bacterial strains and the rate of diffusion of the antimicrobial agent in agar. In practice, the results from disk diffusion test are interpreted by comparing the value of inhibition zone with criteria published by the CLSI. Thus, the samples are classified as sensitive, intermediate or resistant.

RESULTS AND DISCUSSION

From the chromatographic evaluation of the *Equisetum arvense* (Ead and Eain) extract, it was observed that the Eain extract (*in natura*) presents a number of polar and medium polar compounds and few non-polar compounds that can be observed under UV-vis irradiation. The chromatographic plates of this extract also show that the polar and medium polar compounds are also revealed with phosphomolybidic acid and ninhydrin, which indicates the presence of compounds with oxygen containing functional groups and amino acids,

respectively. On the other hand, the Ead extract (dehydrated plant), presents compounds of only medium polarity that can be observed under UV-vis irradiation. These compounds reveal only with phosphomolybidic acid. Analyzing these results it can be seen that the dehydration process leads to a loss of the polar compounds that correspond partially to primary metabolites (amino acids), but that can also contain secondary metabolites that are important to the biological activity of the plant.

From the evaluation of the chromatographic extracts of green tea (Cv.1, Cv.2 and Cv.3) it can be observed that these present the same composition of secondary metabolites. Thus, the dehydration process employed, apparently, does not lead to the loss of active components. The green tea extracts present polar compounds, medium polar compounds and non-polar compounds that reveal under UV-vis irradiation. The extracts Cv.1, Cv.2 and Cv.3 present medium polar compounds and non-polar compounds that reveal are also revealed with phosphomolybid acid and Dragendorff reagent, which demonstrates the presence of compounds with oxygen containing functional groups and alkaloids, respectively. The chromatographic plates of the green tea extracts also indicate the presence of polar amino acids that are revealed by ninhydrin.

The *Mikania glomerata* extract (Mg) presents medium polar compounds and non-polar compounds that are revealed under UV-vis irradiation and with phosphomolybid acid, which indicates the presence of compounds with oxygen containing functional groups. The *Ginkgo biloba* extract (Gb), presents a number of polar and medium polar compounds and few non-polar compounds that can be observed under UV-vis irradiation and that also are revealed by phosphomolybid acid.

The NMR-^{1}H spectra of the plant extracts under study can be seen in Figure 1. For the *Equisetum arvense* extracts (Ead and Eain), it can be observed that there is a difference in their composition. This can be observed by first considering the spectrum of the sample *in natura* (Eain) that presents the following characteristics: methylene hydrogens (R-CH_2-R) between δ 1,0-1,5 ppm, signals of hydrogens bonded to functionalized carbons between δ 3,0-4,0 ppm, a signal at δ 3,6 ppm characteristic of methoxyl group hydrogens (-OCH_3), signals of hydrogens bonded to double bonded carbons between δ 4,0-6,0 ppm and less intense signals between δ 6,0-8,0 ppm that are characteristic of hydrogens bonded to an aromatic ring. The spectrum of the dehydrated plant does not display as many signals of hydrogens bonded to double bonded carbons. In addition, the signals of hydrogens bonded to aromatic rings are practically absent.

The NMR-^{1}H spectra of the green tea extracts (Gt.1, Gt.2 and Gt.3) display the same pattern of methylene hydrogen (R-CH_2-R) signals between δ 1,0-1,5 ppm and signals of hydrogens bonded to functionalized carbons between δ 3,0-4,0 ppm. The green tea extracts present at least 3 signals at δ 3,4, δ 3,5 e δ 3,8 ppm that are characteristic of methoxyl (-OCH_3) group, hydrogens. The spectra also present signals of hydrogens bonded to double bonded carbons between δ 4,0-6,0 ppm and various signals between δ 6,0-8,0 ppm that are characteristic of hydrogens bonded to aromatic rings.

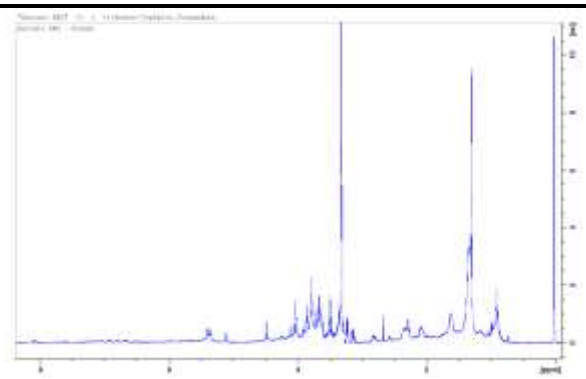
The *Ginkgo biloba* (Gb) extract`s NMR-^{1}H spectrum presents an intense signal of methylene hydrogens (R-CH_2-R) between δ 1.0-1.5 ppm, signals of hydrogens attached to carbons functionalized between δ 3 0-4.0 ppm, a signal at δ 3.4 ppm characteristic of hydrogens methoxyl group (-OCH_3), signals of hydrogens attached to carbon in a double bond between δ 4.0-6.0 ppm, besides some signals at δ 6.5-7.5 ppm characteristic of hydrogens attached to the aromatic ring. Yet, the spectrum NMR-^{1}H of the extract of *Mikania glomerata* (Mg) has several signals of hydrogens at methyl groups (R-CH_3) and methylene (R-CH_2-R) between δ 1.0-1.5 ppm signals of hydrogens attached to carbons functionalized between δ 3.0-4.0 ppm, a very intense signal at δ 3.4 ppm characteristic of hydrogens from a methoxyl group (-OCH_3), signals from hydrogens attached to carbons in a double bond between δ 4.0-6.0 ppm and various signals of δ 6.0-8.0 ppm characteristic of hydrogens attached to the aromatic ring.

From the pharmacological evaluation of bioassays using antimicrobial activity, it appears that the *Equisetum arvense* extracts from the dried plant that can be bought in shops of the city and is widely used by the population has an intermediate biological activity against *E.coli* than the fresh plant. However the extracts of the dried and the fresh plant are sensitive against *S. aureus.* On the other hand, both extracts are resistant against the fungi tested.

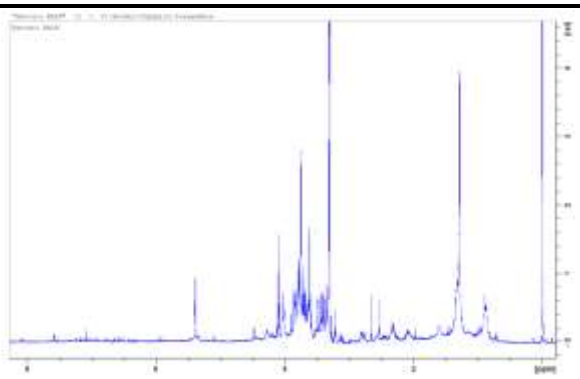
As for the green tea, extracts 2 and 3 are sensitive against *S. aureus* and against both fungi tested. However, with respect to *E. coli* all extracts showed intermediate biological activity. This demonstrated that the industrial dehydration process does not follow the same rules and that the brand products 2 and 3 are more recommended for consumption as they present better results for biological activity.

With respect to plants *Mikania glomerata* and *Ginkgo biloba* it was verified that both have an intermediate antimicrobial activity against the tested Candida albicans and *A. flavus*, respectively. As both plants studied were commercially purchased in dehydrated form and have not been studied *in natura* it was not possible to say whether the process of dehydration leads to the loss of important secondary bioactive metabolites for the expression of biological

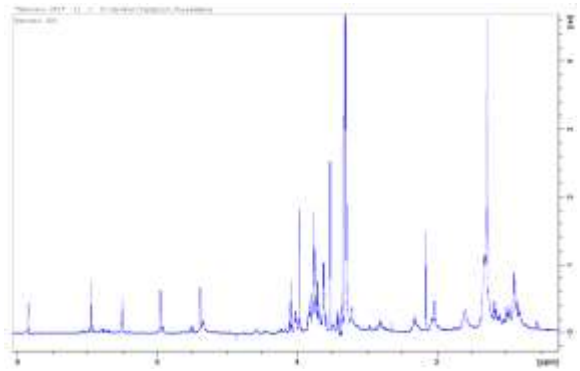
activity. Future studies should be made for this purpose



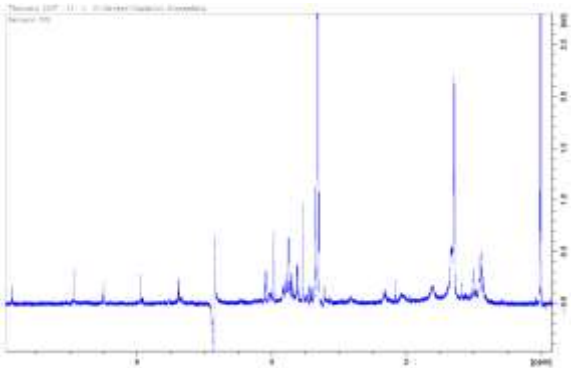
NMR-^{1}H of the Extract Ead



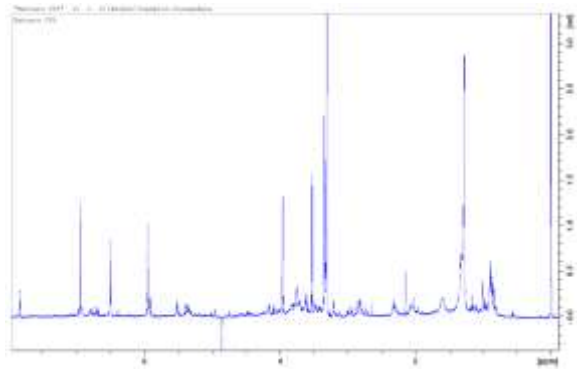
NMR-^{1}H of the Extract Eain



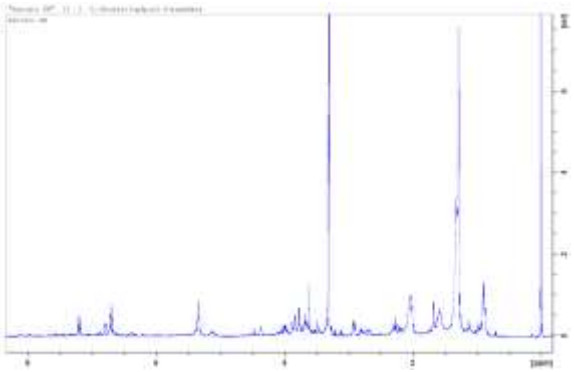
NMR-^{1}H of the Extract Gt.1



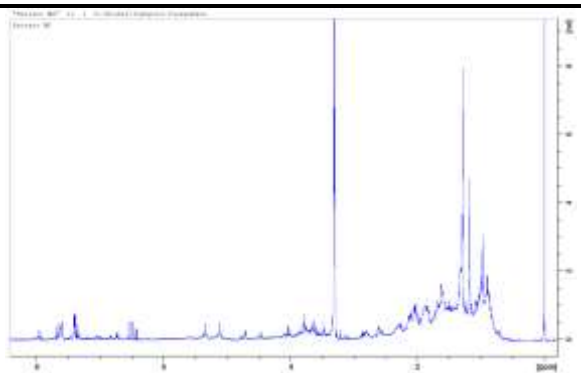
NMR-^{1}H of the Extract Gt.2



NMR-^{1}H of the Extract Gt.3



NMR-^{1}H of the Extract Gb



NMR-^{1}H of the Extract Mg

Figure 1 - RMN-^{1}H Spectrums of the crude extracts of the plants studied.

Table 1 - Bioassays activity antimicrobial data with the extracts studied.

Extrato	***E. coli***	***S. aureus***	***A. flavus***	***C. albicans***
		Inhibition halo (mm)		
Eain	12.28	21.2	9.6	10.6
Ead	6.17	26.2	4.6	-
Mg	1.05	1.35	-	14.0
Gb	7.1	4.6	17.1	13.4
Gt.1	13.5	13.0	-	12.0
Gt.2	14.9	20.5	23.7	18.3
Gt.3	13.9	26.5	21.8	15.8

CONCLUSIONS

The screening performed with some medicinal plants used by the people of the Triangulo Mineiro region - MG was important because it was possible to map the chemical and pharmacological pattern of the plant extracts. From this study it was possible to verify that two different brands of green tea are sensitive against the *S. aureus* and both fungi tested. Also, it was possible to verify that Gb and the Mg extracts are sensitive against *A. flavus* and *C albicans*, respectively while the Eain extract is active against *S. aureus.* This is the first step in the study of bioassay-guided isolation of secondary metabolites of these plants. The bioassay-guided isolation of the secondary metabolites is important because this type of study saves time, stationary phases and organic solvents also involved in the separation and purification process and is therefore considered a "Green Chemical Process".

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