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Research Article

The Effect of Antioxidant activity, Total Phenols and Total Flavonoids on Arginase Inhibitory Activity on Plants of Genus *Sterculia*

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ABSTRACT

Background: The genus of *Sterculia* has the main compound of phenol and flavonoids. The secondary metabolites which have an arginase inhibitory activities were phenol and flavonoids. The aim of this study was to investigate the arginase inhibitory activity from genus *Sterculia*. The Plant of *Sterculia*: *Sterculia rubiginosa* Zoll. ex Miq., *Sterculia comosa* (Wall) Roxb., *Sterculia parkinsonii* F. Muell, *Sterculia macrophylla* Vent, *Sterculia Stipulata* Korth. The simplisia were leaves and woods. **Materials and Methods:** The simplisia were extracted with n-hexane, ethyl acetate and methanol. The ethyl acetate and methanol extract determined the arginase inhibition activity. The active extracts as an arginase inhibitory, determined the total flavonoids, total phenols and antioxidant activity, and the chemical content. *Sterculia comosa* (Wall) Roxb., *Sterculia macrophylla* Vent, *Sterculia Stipulata* Korth., have arginase inhibitory activity. **Results:** The ethyl acetate extracts of *Sterculia Stipulata* leaves is an active extract. The methanol extract which have an arginase inhibitor activity were *Sterculia comosa* (Wall) Roxb. wood and leaves, *Sterculia macrophylla* Vent., wood and leaves, *Sterculia stipulata* Korth., wood, and leaves. The methanol extract of *Sterculia comosa* (Wall) Roxb. Woods has the highest content of total phenols, antioxidant activity, and arginase inhibitory activity. The methanol extract of *Sterculia macrophylla* Vent. has the highest content of total flavonoids, but this extract as an arginase inhibitory activity more lower than *Sterculia comosa*. The active extract as an arginase activity was methanol extract of *Sterculia comosa* (Wall) Roxb. **Conclusion:** The total phenols were more contributed for the response of the arginase inhibitory activity much more than antioxidant activity and total flavonoids.

Key words: Arginase, Antioxidant, Enzyme, Flavonoids, Phenols, *Sterculia*.

INTRODUCTION

The genus of *Sterculia* was included in the subfamily of *Sterculioideae*, the family of *Malvaceae* and right now becomes the family of *Sterculiaceae*,[1] *Sterculia* consists of 200 species. The stem wood, leaves, fruit, and roots of the *Sterculia* have been used traditional medicine in many countries to treat various diseases, including digestive diseases, diabetes, respiratory diseases, and skin diseases. In addition, the genus *Sterculia* has been studied and has activities as antimicrobial, anti-inflammatory, antioxidant and anticancer[2] cytotoxic and immunomodulatory activities,[3] anti-nociceptive and anti-inflammatory,[4] sedative[5] antibacterial[6] and anti-TB.[7] The genus *Sterculia* contains of compounds flavonoids and their derivatives, terpenoids mostly as triterpenoids, coumarins, alkaloids and other groups such as phenolic acid, phenyl propanoid, fatty acids, sugar and some steroids.[8] The literature study confirms that the main content of the genus *Sterculia* was flavonoids which include flavones, C-glycoside flavones, flavonols, flavan, isoflavones, isoflavan and anthocyanins. Other phenolic compounds such as phenolic acid, propanoid phenyl, coumarin, lignans and lignin.[2] Indonesia has plants of genus *Sterculia*: *Sterculia macrophylla* Vent. was found in Sumatra, Maluku and Papua. *Sterculia rubiginosa* Zoll. ex Miq. was found in Sumatra. *Sterculia parkinsonii* F. Muell was found in Papua. *Sterculia stipulata* Korth and also *sterculia comosa*. Arginase was an enzyme responsible for converting L-arginine to L-ornithine and urea.[33] The substrat was L-arginine. This substrat used for Arginase and nitric oxide synthase (NOS), they use same substrat, so arginase competes with NOS for arginine.[9,10] Nitric oxide (NO) production has been correlated to arginase activity in vessels, such as in physiological and pathological conditions on hypertension,[11] diabetes,[12,13] erectile dysfunction,[14] atherosclerosis,[15,16] and endothelial disfunction.[16] Some secondary metabolites have arginase inhibitory activity such as phenol and flavonoids.[18] It was interesting to find the relationship between total phenols, total flavonoids and antioxidant activity with the inhibitory enzyme arginase on *Sterculia*.

MATERIALS AND METHODS

Materials

The *Sterculia* genus used were: *macrophylla* Vent, *Sterculia stipulata*, *Sterculia parkinsonii*, *Sterculia comosa* and *Sterculia rubiginosa*. The part of the plant from *Sterculia* used were leaves and woods. The Plants collected from Botanical Garden of Bogor, Indonesia and determined in Botany Herbarium Research Institute, Cibinong, West Java. The solvents used were n-hexane, ethyl acetate and methanol from local suppliers. Nor-NOHA (N$^{\omega}$-hydroxy-L-arginine) standard (Cayman, USA). Arginase enzymes (Sigma, Singapore), maleic acid (Sigma, Singapore). DMSO (Dimethyl sulfoxide) (Merck, Germany) and L-arginine (Sigma, Singapore). Ethyl acetate pro-analysis (Merck, Germany),

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methanol pro analysis (Merck, Germany), n-hexane pro analysis (Merck, Germany), manganese sulfate (Sigma, Singapore). Urea assay kits (Quantichrom® Bioassay, United States), DPPH (2,2-Diphenyl-1-picrylhydrazyl) (Merck, Germany), the chemical reagents for identification of the compound and determining the content phenols total and flavonoids total.

Extraction

The powder from woods and leaves of *Sterculia* plants (20 g) were extracted by using a solvent continously with n-hexane, ethyl acetate and methanol. The ratio between powder and solvent was 1:10. Each extract was concentrated with a rotary evaporator at 50 °C, then continued using a waterbath at 50 °C. Ethyl acetate and methanol extracts were tested for their activity as an arginase inhibitor.

Arginase inhibitor activity

The method for determined the arginase inhibitor activity used the procedure from the Kit and has been slightly modified. This procedure has also been carried out in previous studies. In the preliminary research, the concentration of the extracts in the well was made 100 µg/ml. The extract (50 mg) was added with 400 µl DMSO to dissolve, added with aquabidestillata to 5 ml (Stock 1). This solution was taken 1 ml and diluted with aquabidestillata to 2 ml (stock 2). From the stock 2, 90 µl was taken and diluted with aquabidestilata to 1 ml (stock 3). This solution (stock 3) would be tested for arginase inhibitory activity. Then (10) µl extracts solution (stock 3) were added to the well, added 15 µl enzyme (1 U/ml) added 25 µl of L-arginine (570 mM) solution and shake for 5 sec. Incubated at 37 °C for 30 min. After incubation added with 100 µl urea kits A and B (1: 1), shake for 5 s. Incubate for 1 h at room temperature. The absorbance was read at 430 nm. The concentration extract for this activity was 100 µg/ml in well. The nor-NOHA as a positive control was performed under the same conditions and determined the IC_{50}.

Antioxidant activity

The Antioxidant activity used the DPPH method from Bobo garcia (2015) with a slight modification.[19] For the antioxidant activity, the concentration of extracts were used 100 µg / ml. Twenty (20) µl extract in methanol added 180 µl DPPH 150 µmol /l solution in methanol, put into the well. The mixture shake for 60 s, incubate for 40 min in a dark place. The absorbance results was read at 515 nm. Methanol was used to replace the extract as a control. Quercetin was used as a positive control. The antioxidant activity was calculated as follow:

$$\text{Inhibition (\%)} = \frac{Absorbance\ control - Absorbance\ sample}{Absorbance\ control} \times 100\%$$

Total phenols

The determination of total phenols content used the method from Farasat (2014) with microplate as an instrument.[20] The concentration of the extract in the well was 100 µg/ml. Twenty (20) µl extracts in methanol (1000 µg/ml) were added in the well, added 100 µl of Folin reagent (Folin-Ciocalteu: destilate water = 1:10). After 4 min, the mixture added 80 µl of Na_2CO_3 7.5%. The incubation was carried out for 2 h in a dark place at room temperature. The absorbance was read at 600 nm. The standard curve was used gallic acid (3.125; 6.25; 25; 50; 100 µg/ml). Total phenols content was calculated as gallic acid equivalent (mg)/gram dry extract (mg GA /g extract).

Total flavonoids

The determination of total flavonoids content used the method from Farasat (2014) with microplate as an instrument.[20] The concentration of the extract in the well was 100 µg/ml. Twenty (20) µl extracts in methanol (1200 µg/ml) were added in the well, 20 µl aluminium chloride 10%, Added 20 µl potassium acetate 1 M and 180 µl distilled water. The mixture was incubated for 30 min at room temperature. The absorbance was read at 415 nm. The standard curve was used quercetine (3; 6; 9; 12; 18; 24 µg/ml). Total flavonoids was calculated as quercetine equivalent (mg)/gram dry extract (mg QE/g extract).

Phytochemical screening and TLC chromatogram

The chemical compounds in the active extracts were determined by the method of Harbone and Indonesian pharmacopoeia.[21,22] The content of the chemical compounds: tannins, alkaloids, flavonoids, phenols, saponins and anthraquinones. The active extracts were determined the profile of TLC chromatogram.

Statistical analysis

The multiple linear regression was used as a statistical analysis to find the relationship between antioxidant activity, total phenols and total flavonoids on arginase inhibitory activity. The total phenols, total flavonoids and antioxidant activity as independent variable, and dependent variable was arginase inhibitory activity.

RESULTS AND DISCUSSION

Arginase activity

Methanol extract was an active extract from plants in the genus *Sterculia*. This active extract in wood and leaves. The results showed in table 1 and table 2. The IC_{50} for nor-NOHA as a positive control was 3.773 µg / ml. The result showed on table 2 and figure 1.

Antioxidant activity

The DPPH was method to determined the antioxidant activity. Quercetine was used as a positive control, the IC_{50} of quercetine was 5.63 µg/ml. The result of antioxidant activity showed on table 3 and figure 2.

Determination of total flavonoids and total phenols

The determination of total flavonoid was used quercetine as a standard. The result of linear regression was y = 0.0198x - 0.0215 (R^2 = 0.9964). *Sterculia macrophylla* leaves extract had the highest of total flavonoids. The total flavonoids was 67.74 mg QE/gram. The determination of total phenol was used gallic acid as standard. The linear regression was: y = 0.026x + 0.3373 (R^2 = 0.996). The highest phenol content was *Sterculia comosa* wood extract. The value was 709.39 mg GAE/gram. The result showed on table 4 and table 5. The active extracts as an arginase inhibitor were determine the total phenols and total flavonoids.

Phytochemical screening

The active extract as an arginase inhibitor was determined the chemical constituents.

The results showed on table 6. The Chromatogram Profile of Active Extracts showed on table 7.

Statistical analysis

The multiple linear regression used for statistical analysis to find the effect of antioxidant activity, total phenols and total flavonoids to arginase inhibitory activity. The Significance value 0.000 (*P<0,05) it was meant that Ho was rejected. It can be said that total phenol, total flavonoids and antioxidant activity have an affect to the arginase inhibitory activity. The value of beta coefficient for total phenol was 0.891; the value of total flavonoid was -0.224 and the value of antioxidant activity was -0.053, it can be concluded that the total phenols more contributed

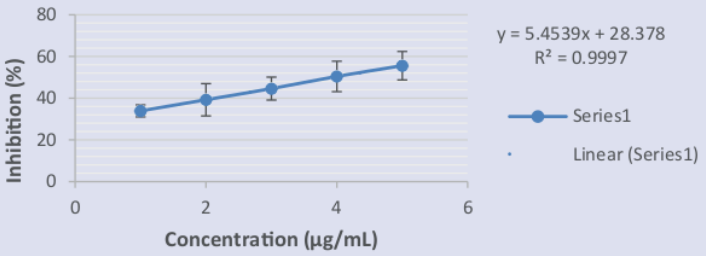

Figure 1: The arginase inhibitor activity of nor-NOHA.

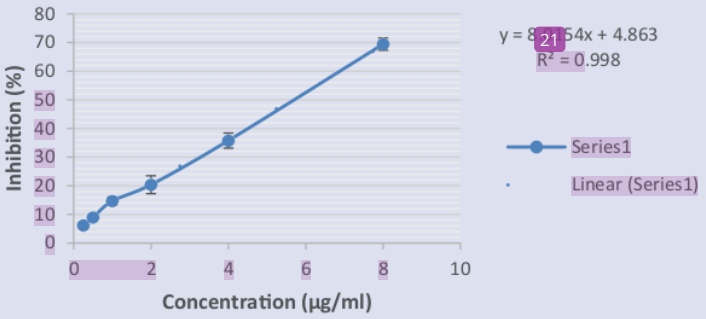

Figure 2: Antioxidant activity of quercetine.

Table 1: Arginase inhibitor activity of methanol extracts.

Extract (100 µg/ml)	Average Inhibision (%)	Sd	kv
Leaves			
Sterculia comosa	61.66	7.07	11.46
Sterculia macrophylla	32.61	5.56	17.07
Sterculia parkinsonii	-92.75	13.71	-14.78
Sterculia rubiginosa	-121.80	5.89	-4.83
Sterculia stipulata	14.47	2.07	14.30
Woods			
Sterculia comosa	84.25	10.34	12.28
Sterculia macrophylla	92.54	5.90	6.38
Sterculia parkinsonii	-66.71	11.41	-17.11
Sterculia rubiginosa	-222.17	17.33	-7.80
Sterculia stipulata	17.80	3.00	16.84
Nor-NOHA (IC_{50})	3.733 µg/ml	R^2=0,9997	

Table 2: Arginase inhibitor activity of ethyl acetate extracts.

Extract (100 µg/mL)	Average Inhibision (%)	Sd	kv
Leaves			
Sterculia comosa	-35.57	6.63	-18.64
Sterculia macrophylla	-93.36	12.61	-13.51
Sterculia parkinsonii	-93.16	14.06	-15.10
Sterculia rubiginosa	-76.56	14.81	-19.34
Sterculia stipulata	-51.03	5.75	-11.27
Woods			
Sterculia comosa	-12.54	0.05	-0.42
Sterculia macrophylla	-8.31	1.35	-16.24
Sterculia parkinsonii	-64.90	11.48	-17.69
Sterculia rubiginosa	-2.96	0.55	-18.50
Sterculia stipulata	19.19	1.25	6.49
Nor-NOHA (IC_{50})	3.733 µg/ml	R^2=0,9997	

Table 3: Antioxidant activity of methanol extracts.

Extra (100 µg / ml)	The part of Plant	Antioxidant Activity (%)	sd	kv
Sterculia stipulata	Leaves	78.81	1.26	1.60
Sterculia macrophylla	Leaves	78.65	2.69	3.42
Sterculia stipulata	Woods	8.30	0.38	4.60
Sterculia macrophylla	Woods	77.20	2.53	3.28
Sterculia comosa	Woods	91.31	1.67	1.83
IC_{50} Quercetine		5.63 µg / ml		

Table 4: Total phenols content of methanol extract.

Extract (100 µg/ml)	The Part of Plant	Total Phenols (mg GAE/g)	sd	kv
Sterculia stipulata	Leaves	141.62	10.54	7.44
Sterculia macrophylla	Leaves	316.29	35.66	11.27
Sterculia stipulata	Woods	50.00	5.15	10.30
Sterculia macrophylla	Woods	515.00	37.33	7.25
Sterculia comosa	Woods	709.39	35.47	5.00

Table 5: Total flavonoids content of methanol extract.

Extract (100 µg/ml)	The part of Plant	Total Flavonoids (mg QE/g)	sd	kv
Sterculia stipulata	Leaves	41.45	5.84	14.08
Sterculia macrophylla	Leaves	67.74	6.50	9.60
Sterculia stipulata	Woods	27.99	0.62	2.22
Sterculia macrophylla	Woods	28.87	4.24	14.69
Sterculia comosa	Woods	33.27	3.74	11.24

Table 6: Phytochemical screening of the methanol extract.

Extract	Sterculia stipulata Leaves	Sterculia macrophylla Leaves	Sterculia comosa Woods	Sterculia macrophylla Woods	Sterculia stipulata Woods
Terpenoids/steroids	Terpenoids +	Terpenoids +	Steroids +	Steroids (+)	Terpenoids +
Alkaloids					
- Dragendorff	+	+	+	+	+
- Mayer	+	+	+	+	+
Tannins					
- $FeCl_3$	+	+	+	+	-
- Folin	+	+	+	+	+
- Gelatine	+	+	+	+	+
Flavonoids	+	+	+	+	+
Antraquinones	-	-	-	-	-
Saponins	+	+	+	+	+

Note: + = presence, - = Absence

Table 7: The chromatogram profile of active extracts.

No.	Mobile phase	Sterculia stipulata Woods (Rf, UV365)	Sterculia stipulata Leaves (Rf, UV365)	Sterculia macrophylla Leaves (Rf, UV365)	Sterculia comosa Woods (Rf, UV365)	Sterculia macrophylla Woods (Rf, UV365)
1	Hexan: Ethyl acetate: Methanol (11:4:2) Stationary phase: Silica Gel GF_{254}	0.76 (red) 0.89 (blue)	0.76 (red) 0.91 (red)	0.36 (blue) 0.67 (black) spray with H_2SO_4 10% becomes yellow 0.76 (red) 0.91 (red)	0.45 (blue) 0.76 (blue fluorescent 0.91 (blue)	0.76 (blue fluorescent) 0.91 (blue)
2	Ethyl acetate: Methanol (9:1) Stationary phase: Silica Gel GF_{254}	0.2 (blue) 0.54 (blue) 0.76 (blue) 0.89 (blue)	0.15 (blue) 0.85 (red) 0.89(blue) 0.91 (red)	0.21 (black) spray with H_2SO_4 10% becomes yellow 0.85 (red) 0.89 (blue) 0.91 (red)	0.22 (light blue) 0.45 (light blue) 0.76 (blue fluorescent) 0.84(blue)	0.45(light blue) 0.76(blue fluorescent) 0.89 (light blue)
3	Ethyl acetate : Methanol : Formic acid (8:3:0,1) Stationary phase: Silica Gel GF_{254}	0.64(light yellow) 0.73 (light yellow) 0.76 (blue fluorescent) 0.91 (light yellow)	0.64(light yellow) 0.73 (yellow) 0.76 (orange) 0.91 (red)	0.55 (blue) 0.69 (black) spray with H_2SO_4 10% becomes yellow 0.76 (yellow) 0.91(red)	0.55 (blue) 0.64 (blue) 0.76 (blue) 0.91 (blue)	0.64 (light yellow) 0.76 (blue fluorescent) 0.91 (blue)

for the arginase activity than total flavonoids and antioxidant activity. The VIF (Variance Inflance *Factor)* values showed for total phenols 2.338, total flavonoids 1.444 and for antioxidant 2.430, from the three independent variables showed that there was no multicolinerity. The multiple linear regression with 3 independent variables as follow: $Y = 28.289 + 0.128\ X_1 - 0.497\ X_2 - 0.069\ X_3$. X_1= independent variable (total phenols), X_2= independent variable (total flavonoids), X_3= independent variable (antioxidant activity).

Endothel dysfunction was related to arginase activity, one of the disease was hypertension. L-arginine was a substrate that used by NOS and arginase. Under physiological conditions NOS maintains the health of blood vessels by producing NO. Arginase produces ornithine, which would be metabolized to polyamine for tissue growth and proline for collagen. Under pathological stimulation with the presence of RhoA/ROCK, arginase activity would be increase so that it would deplete the substrate NOS, L-arginine. When NOS does not have enough substrate, it will become unbound and produce more superoxide (O^{2-}) than NO. Increased production of polyamines and proline can also cause pathological and vascular stiffness.[23,24] Availability of NO will affect the regulation of vascular tone and maintenance of vascular integrity.[2] The inhibition of arginase activities by phenol, flavonoids, and some of them were chlorogenic acid, quercetine, epicatechin, wogonin, (2R, 4S) -4,5,6,7,8,4′-Hexamethoxylflavan, (2S)5, 7, dihydroxy8,2′dimethoxyflavanone, (2S)-5, 2,5′-Trihydroxy-7,8-dimethoxyflavanon, naringenin, 7-Hydroxysauchinone, taxifolin, kaempherol, caffeic acid, Sauchinone, meso-Dihydroguaiaretic acid, apigenin, resveratrol, piceatannol, Guaiacin, Naringenin-5-O-β- D-glucopyra. (2S) 5,7-Dihydroxy-7,8 dimethoxyflavanone-2′-O-β-D-glucopyranoside.[25] Flavonoids such as luteolin, fisetin can inhibit the arginase enzyme.[26] Our previous study showed that *Sterculia macrophylla* which has arginase activity also have high of antioxidant activity and total flavonoids.[27] The woods of *Sterculia* more active than leaves. And the methanol extract more active than ethyl acetate extract for inhibit arginase. The recent study the stem bark of *Caesalpini turtuosa* have the arginase activity with the IC_{50} 11.58 µg/ml for methanol extract and 33.81 µg/ml for ethyl acetate extract.[28] This result was same with our study. *Sterculia* contain phenol compounds and flavonoids as the abundant compound.[1] Interesting to examine whether the arginase inhibitory activity of from the genus Sterculia was influenced by antioxidant activity, total phenol levels and total flavonoids. The analytical results by multilinear regression analysis showed that total phenol was more contributed for this activity than total flavonoids and antioxidant activity. *Sterculia comosa* and *Sterculia macrophylla* have a high inhibitory activity on arginase. It is need more research to know the chemical compound which was responsible for this activity.

CONCLUSION

The total phenols of the plant of *Sterculia* genus responsible for the arginase inhibitory activity. The most active plants was *Sterculia comosa* woods. Based on this study *Sterculia comosa* woods may be used for many diseases causes by endothelial dysfunction.

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CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

ABBREVIATIONS

S: *Sterculia*

TPTZ: 2,4,6-tripyridyl-s-triazine

FRAP: Ferric Reducing Antioxidant Power

AFS: Ammonium ferrous sulphate

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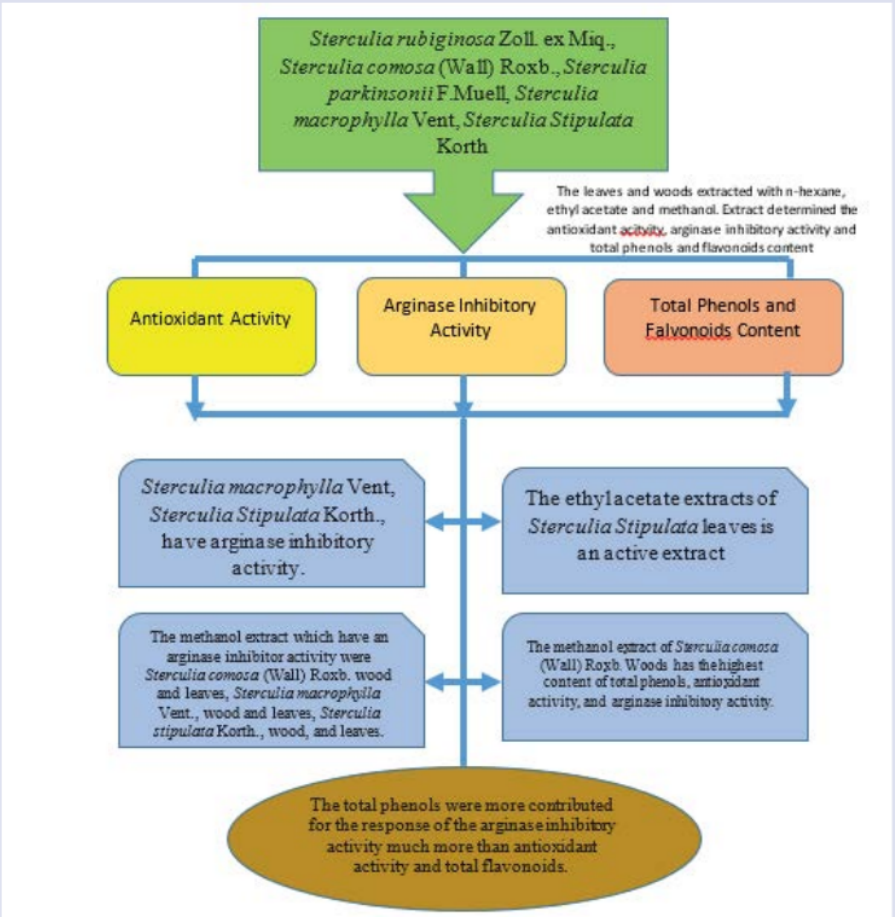

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