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COMPARATIVE STUDY OF THE EFFECTS OF *LAURUS NOBILIS* AND *SYZYGIUM AROMATICUM* AQUEOUS EXTRACTS ON URINE VOLUME AND RENAL FUNCTION IN RATS

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ABSTRACT: The purpose of the current study was to investigate the diuretic and renal effect of the aqueous extracts of *Laurus nobilis* leaves and dried flower buds of *Syzygium aromaticum*. Four groups of rats were used. Group 1 received distilled water (10 ml/kg BW), group 2 received the aqueous extract of *Laurus nobilis* (300 mg/kg BW), group 3 received the aqueous extract of *Syzygium aromaticum* (300 mg/kg BW) and group 4 received furosemide (15 mg/kg BW). The interventions were administered daily for nine days. The urinary volume was measured every day. On day nine, the kidneys were removed for histological analysis, the urine and blood samples were obtained from each rat to analyze urine and plasma potassium, sodium, chloride and creatinine, serum urea, and serum albumin. Renal clearance, saluretic activity, estimation of the carbonic anhydrase inhibition activity, osmolar clearance, plasma osmolarity, free water clearance, free water reabsorption were calculated. The results showed that furosemide and both plants extract produced a significant increase in diuresis, creatinine clearance, and saluretic index, furosemide increase significantly the urinary excretion of Na⁺, K⁺ and Cl⁻ while both plants increased Na⁺ and Cl⁻ and a less effect on potassium excretion without causing hypokalemia or changing blood urea, albumin, blood creatinine, urine creatinine, and natriuretic index. While furosemide caused hypokalemia. No alteration was shown in the kidney structure of all groups.

INTRODUCTION: Diuretics are drugs that increase the production of urine; they are used to control fluid overload and to treat edema, nephritic syndrome, heart failure hypertension, and cirrhosis.

While the majority of the available diuretics are associated with several side effect like hyponatremia, hypokalemia and metabolic abnormalities, which requires scientists to not stop looking for other bioactive molecules with diuretic properties¹.

Herbal medicine refers to the use of plants in the treat of illnesses. A plant is qualified as medicinal when some of its organs have medicinal properties². The traditional uses of medicinal plants have long histories; it present in all ancient civilizations.

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Despite medicine's progress, the traditional use of the medicinal plant is very present, and the use of plants as diuretics is among the most widely uses³. *Syzygium aromaticum* (clove) is a tree (10-12m high) from the Myrtaceae family. The main producers of clove are from Indonesia, India, Malaysia, and Sri Lanka⁴. Clove is very rich in essential oil; the major compound of this oil is eugenol, which is much known for these beneficial effects for health⁵. The traditional use of *Syzygium aromaticum* dates from the far past by dint of the wide range of pharmacological effects of this medicinal plant such as Antioxidant activity, Antimicrobial activity, Antinociceptive, Antiviral, and anti-proliferative activity⁶.

Laurus nobilis (bay) is an evergreen tree belongs to the Lauraceae family. Traditionally, the leaves of *Laurus nobilis* are used as a culinary condiment, diuretic, carminative, and to treat rheumatism. Pharmacologically, the aqueous extract of the laurel is endowed with a healing activity⁷ reduced glycemia and improve the lipid profile in type 2 diabetics⁸, bay essential oil has been shown to be antibacterial, antioxidant and antifungal, it is used for food preservation⁹ and as a bioinsecticide¹⁰. This study aims to contribute to the growing area of research in medicinal plants by exploring the effect of *Syzygium aromaticum* and *Laurus nobilison* diuresis and renal function in normal Wistar rats.

MATERIALS AND METHODS:

Plant Collection and Extraction: The leaves of *Laurus nobilis* were harvested in April-May 2016 from Kenitra in the North-west of Morocco (voucher specimen: LAR 15-2291), and the dried flower buds of *Syzygium aromaticum* were bought from an herbalist from Dakhla in the southern of Morocco (voucher specimen: DAK 14-1610). The botanical identification of the species was carried out by Professor Amina Bari, botanist in the Department of Biology, Faculty of Sciences Dhar Mahraz, University Sidi Mohammed Ben Abdellah, Fez, Morocco. Twenty grams of each plant were boiled with 250 ml of distilled water under reflux during 10 min; the decoction obtained was filtered and stored at 4 °C until use.

Experimental Animals: Our experiments were performed on normal male wistar rats (n = 12), bodyweight of 200 ± 20 g. The animals stayed in a

room with a temperature of 25 ± 2 °C, and the light imposed from 6 h to 18 h. Rats received daily and freely water and food. The care and use of animals were in accordance with Directive 86/609/EEC¹¹.

Experimental Design: The rats were divided into four groups of three rats each: Group 1: received distilled water at a rate of 10 ml/kg of the body weight; Group 2: received by gavage the aqueous extract of *Laurus nobilis* at a rate of 300 mg/kg of the body weight; Group 3: received by gavage the aqueous extract of *Syzygium aromaticum* at a rate of 300 mg/kg of the body weight; Group 4: received by gavage furosemide (Lasilix), reference drug at a dose of 15 mg/kg of the body weight. The dose of *Laurus nobilis* and *Syzygium aromaticum* were similar to the doses used in rats^{12,13}.

The diuresis of the rats is followed for nine days, the urine of 24 h was collected daily, the volume obtained for each rat was recorded, and a sample was obtained and stored for analysis. At the end of the experiment, the rats were sacrificed under total anesthesia, and blood samples were collected from each rat, and the kidneys were removed and fixed directly in formalin solution 10%.

Biochemical Analysis: The urine obtained from each rat was analyzed for sodium, potassium, chloride and creatinine content, while the serum of each rat was analyzed for sodium, potassium, chloride, creatinine, urea and albumin. And the following parameters were calculated: Glomerular filtration rate = $\text{Ucreatinine} \times \text{V/Pcreatinine}$; Natriuretic activity = Na^+/K^+ ; Saluretic activity = $\text{Na}^+ + \text{Cl}^-$; Estimation of the carbonic anhydrase inhibition activity = $[\text{Cl}^- / \text{Na}^+ + \text{K}^+]$; Osmolar clearance = $\text{Urinary osmolarity} \times \text{V} / \text{plasma osmolarity}$; Plasma osmolarity = $2 \times \text{Na}^+$; Urinary osmolarity = $2 \times (\text{Na}^+ + \text{K}^+)$; Free water clearance = $\text{Urine flow} \times (1 - \text{urinary osmolarity} / \text{Plasma osmolarity})$; Free water reabsorption (TCH₂O) = $-\text{CH}_2\text{O}$.

Histological Analysis: Samples of the kidneys which were already fixed in 10% formalin during 48 h, were dehydrated through the passage in increasing concentration of ethanol bath and clarified in toluene, and then they were included in paraffin.

The histological sections were ruled out by a microtome (5-6 mm) and then stained with hematoxylin and eosin to prepare them for observation under an optical microscope.

Statistical Analysis: Graph pad prism Software was used for Statistical analysis; data were represented as mean \pm SD. Comparison between groups was performed using ANOVA, followed by Tukey's multiple comparison tests, $p < 0.05$ was considered significant throughout the analysis.

RESULTS:

Effect of the Interventions on Urine Volume in Normal Rats in Normal Rats: As shown in **Table 1** the urine volume of rats treated with furosemide, *Syzygium aromaticum*, and *Laurus nobilis* increased significantly from the first day and continued to increase daily during 9 days. In the last days, the diuresis of rats received *Syzygium aromaticum* was similar to the group of rats received furosemide and more significant ($P < 0.05$) than those received *Laurus nobilis*.

TABLE 1: EFFECT OF SYZYGIUM AROMATICUM, LAURUS NOBILIS AND FUROSEMIDE ON URINE VOLUME (ML/24H) IN NORMAL RATS DURING NINE DAYS

	Distilled water	Furosemide	<i>Syzygium aromaticum</i>	<i>Laurusnobilis</i>
Day 1	5.59 \pm 0.37** ^b	7.75 \pm 1.20** ^a	7.45 \pm 1.15* ^a	7.50 \pm 0.50* ^a
Day 2	5.42 \pm 0.37*** ^b	8.00 \pm 1.03*** ^a	7.95 \pm 1.06*** ^a	7.75 \pm 0.55** ^a
Day 3	6.08 \pm 0.38*** ^b	10.41 \pm 0.44*** ^a	7.50 \pm 0.25*** ^a *** ^b	7.5 \pm 0.5*** ^a *** ^b
Day 4	6.08 \pm 0.39*** ^b	11.00 \pm 0.81 *** ^a	8.25 \pm 1.18** ^a ** ^b	8.33 \pm 1.24* ^a ** ^b
Day 5	6.07 \pm 0.60*** ^b	14.35 \pm 1.67*** ^a	9.16 \pm 1.06** ^a ** ^b	8.50 \pm 0.91* ^a ** ^b
Day 6	6.37 \pm 0.76*** ^b	14.18 \pm 1.31*** ^a	9.5 \pm 1.5** ^a *** ^b	8.58 \pm 0.83* ^a *** ^b
Day 7	6.31 \pm 0.48*** ^b	14.95 \pm 0.68*** ^a	10.5 \pm 1.5*** ^a *** ^b	10 \pm 1*** ^a *** ^b
Day 8	6.37 \pm 0.76*** ^b	14.66 \pm 0.94*** ^a	13.25 \pm 1.28*** ^a *** ^c	10.33 \pm 0.94*** ^a *** ^b
Day 9	6.38 \pm 0.48*** ^b	14.95 \pm 0.68*** ^a	14.16 \pm 1.21*** ^a * ^c	11.16 \pm 1.21*** ^a *** ^b

a: comparison with the group received distilled water ; b: comparison with the group received furosemide; c: comparison between the group received *Syzygium aromaticum* and the group received *Laurus nobilis*. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

TABLE 2: EFFECT OF SYZYGIUM AROMATICUM, LAURUS NOBILIS AND FUROSEMIDE ON URINE ELECTROLYTES LEVEL IN NORMAL RATS AFTER NINE DAYS OF TREATMENT

Urine electrolytes		Distilled water	Furosemide	<i>Syzygium aromaticum</i>	<i>Laurus nobilis</i>
Urine sodium (meq/L)	Baseline	151.88 \pm 1.3	151.18 \pm 0.75	152.12 \pm 0.21	150.19 \pm 1.22
	Day 9	152.66 \pm 2.05*** ^b	207 \pm 2.51 *** ^a	189.16 \pm 1.34*** ^a *** ^b *** ^c	180 \pm 1.15*** ^a *** ^b
Urine potassium (meq/L)	Baseline	71 \pm 0.44	70 \pm 1.22	71 \pm 0.34	71 \pm 0.33
	Day 9	71.66 \pm 1.24** ^b	84.33 \pm 6.67** ^a	80.66 \pm 5.82 * ^a	81.33 \pm 3.14 * ^a
Urine chloride (meq/L)	Baseline	99 \pm 0.10	98 \pm 0.80	99.10 \pm 0.40	98.15 \pm 0.95
	Day 9	98.83 \pm 0.68*** ^b	206.5 \pm 3.25*** ^a	212.08 \pm 10.64*** ^a	272.2 \pm 1.29*** ^a *** ^b *** ^c

a: comparison with the group received distilled water ; b: comparison with the group received furosemide; c: comparison between the group received *Syzygium aromaticum* and the group received *Laurus nobilis*. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

TABLE 3: EFFECT OF SYZYGIUM AROMATICUM, LAURUS NOBILIS AND FUROSEMIDE ON SERUM ELECTROLYTES LEVEL IN NORMAL RATS AFTER NINE DAYS OF TREATMENT

Urine electrolytes		Distilled water	Furosemide	<i>Syzygium aromaticum</i>	<i>Laurus nobilis</i>
Serum sodium (meq/L)	Baseline	144.20 \pm 0.11	143.00 \pm 1.02	143.10 \pm 0.14	143.80 \pm 0.75
	Day 9	143.50 \pm 1.89	144.83 \pm 0.68	143.66 \pm 0.94	144.5 \pm 0.76
Serum potassium (meq/L)	Baseline	5.10 \pm 0.11	5.20 \pm 0.40	5.09 \pm 0.12	5.12 \pm 0.41
	Day 9	5.07 \pm 0.45	5.16 \pm 0.16	5.23 \pm 0.09	5.14 \pm 0.44
Serum chloride (meq/L)	Baseline	90.45 \pm 1.22	91.00 \pm 1.45	90.45 \pm 1.20	91.00 \pm 0.26
	Day 9	91.55 \pm 1.23	92.83 \pm 2.67	89.83 \pm 4.81	88.60 \pm 4.29

a : comparison with the group received distilled water ; b: comparison with the group received furosemide; c: comparison between the group received *Syzygium aromaticum* and the group received *Laurus nobilis*. (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$)

Effect of the Interventions on Urine Electrolytes Level in Normal Rats: Urinary excretion of sodium and chloride was increased significantly by *Syzygium aromaticum* extract and by *Laurus nobilis* extract. A slight effect was shown on

potassium excretion by both plants. Interestingly the results indicated that *Syzygium aromaticum* and *Laurus nobilis* extracts did not show any significant increase in serum electrolytes, while furosemide causes hypokalemia **Table 2** and **3**.

Effect of the interventions on Blood Urea, Creatinine, Albumin and Glomerular Filtration Rate in Normal Rats: The results summarized in **Table 4** did not show any significant increase in

blood urea, albumin, blood creatinine, and urine creatinine, whereas the clearance of creatinine was increased by furosemide and by both plants extracts.

TABLE 4: EFFECT OF SYZYGIUM AROMATICUM, LAURUS NOBILIS AND FUROSEMIDE ON BLOOD UREA, CREATININE, ALBUMIN AND GLOMERULAR FILTRATION RATE IN NORMAL RATS AFTER NINE DAYS OF TREATMENT

	Distilled water	Furosemide	<i>Syzygium aromaticum</i>	<i>Laurus nobilis</i>
Blood urea(mg/dL)	27.35 ± 1.70	25.5 ± 2.30	24.55 ± 2.75	24.05 ± 5.58
Albumin (g/L)	39.05 ± 0.07	38.5 ± 0.7	36.25 ± 0.77	36.85 ± 1.2
Blood creatinine (mg/dL)	0.42 ± 0.01	0.40 ± 0.01	0.39 ± 0.01	0.38 ± 0.01
Urine creatinine (mg/dL)	46 ± 1.41	45.75 ± 0.35	44.98 ± 1.39	45.82 ± 0.24
Glomerular filtration rate (mL/min)	0.48 ± 0.01*** ^b	1.18 ± 0.05*** ^a	1.15 ± 0.04*** ^{a*c}	0.93 ± 0.03*** ^{a**b}

a: comparison with the group received distilled water; b: comparison with the group received furosemide; c: comparison between the group received *Syzygium aromaticum* and the group received *Laurus nobilis*. (**P < 0.01; ***P < 0.001)

Effect of the Interventions on Urine Osmolarity, Plasma Osmolarity, Osmolar Clearance, Clearance of Free Water and Free Water Reabsorption in Normal Rats: The effect of *Syzygium aromaticum*, *Laurus nobilis* and furosemide on urine osmolarity, plasma osmolarity, osmolar clearance, clearance of free water and free

water reabsorption in rats after nine days of treatment, are summarized in **Table 5**. The results showed a significant increase resulted in urine osmolarity, osmolar clearance, clearance of free water, and free water reabsorption. In contrast, no significant change was registered in plasma osmolarity.

TABLE 5: EFFECT OF SYZYGIUM AROMATICUM, LAURUS NOBILIS AND FUROSEMIDE ON URINE OSMOLARITY, PLASMA OSMOLARITY, OSMOLAR CLEARANCE, CLEARANCE OF FREE WATER AND FREE WATER REABSORPTION IN NORMAL RATS AFTER NINE DAYS OF TREATMENT

	Distilled water	Furosemide	<i>Syzygium aromaticum</i>	<i>Laurus nobilis</i>
Uosm (mOsm/kgH ₂ O)	448.66 ± 4.13*** ^b	582.66 ± 14.45*** ^a	539.66 ± 11.41*** ^{a**b*c}	522.66 ± 6.77*** ^{a**b}
Posm (mOsm/kgH ₂ O)	287.00 ± 4.14	289.66 ± 1.50	287.33 ± 2.06	289.00 ± 1.67
Cosm (mL/min)	7.27 ± 0.63*** ^b	20.87 ± 0.91*** ^a	18.46 ± 1.66*** ^{a *b ***c}	14.03 ± 1.75*** ^{a ***b}
CH ₂ O (mL/min)	-2.62 ± 0.27*** ^b	-10.49 ± 0.51*** ^a	-8.62 ± 0.80*** ^{a**b***c}	-6.27 ± 0.83*** ^{a ***b}
TCH ₂ O (mL/min)	2.62 ± 0.27*** ^b	10.49 ± 0.51*** ^a	8.62 ± 0.80*** ^{a**b***c}	6.27 ± 0.83*** ^{a ***b}

a: comparison with the group received distilled water ; b: comparison with the group received furosemide; c: comparison between the group received *Syzygium aromaticum* and the group received *Laurus nobilis*, (*P < 0.05; **P < 0.01; ***P < 0.001)

Effect of the Interventions on Carbonic Anhydrase Inhibition and on Saluretic and Natriuretic Activities, in Normal Rats: According to **Table 6**, a significant increase was observed in natriuretic and saluretic activities in the

group treated with furosemide while the groups treated with *Syzygium aromaticum* extract and *Laurus nobilis* extract showed a significant increase in saluretic activity and no significant increase in natriuretic activity.

TABLE 6: EFFECT OF SYZYGIUM AROMATICUM, LAURUS NOBILIS AND FUROSEMIDE ON CARBONIC ANHYDRASE INHIBITION AND ON SALURETIC AND NATRIURETIC ACTIVITIES, IN NORMAL RATS AFTER NINE DAYS OF TREATMENT

	Distilled water	Furosemide	<i>Syzygium aromaticum</i>	<i>Laurus nobilis</i>
Saluretic (Na ⁺ + Cl ⁻)	251.50 ± 2.07*** ^b	413.50 ± 3.98*** ^a	401.25 ± 12.74*** ^{a*b}	452.06 ± 2.06*** ^{a**b***c}
Natriuretic (Na ⁺ /K ⁺)	2.13 ± 0.06*** ^b	2.47 ± 0.22*** ^a	2.35 ± 0.20	2.21 ± 0.09
CAI [Cl ⁻ /(Na ⁺ + K ⁺)]	0.44 ± 0.04*** ^b	0.70 ± 0.01*** ^a	0.78 ± 0.04*** ^{a**b}	1.04 ± 0.1*** ^{a**b***c}

a: comparison with the group received distilled water; b: comparison with the group received furosemide; c: comparison between the group received *Syzygium aromaticum* and the group received *Laurus nobilis*. (*P < 0.05; **P < 0.01; ***P < 0.001)

Effect of the Interventions on Kidney Tissue in Normal Rats: Fig. 1 showed that no pathological

change in the kidney tissue was caused by aqueous extracts of *Syzygium aromaticum* or *Laurus nobilis*.

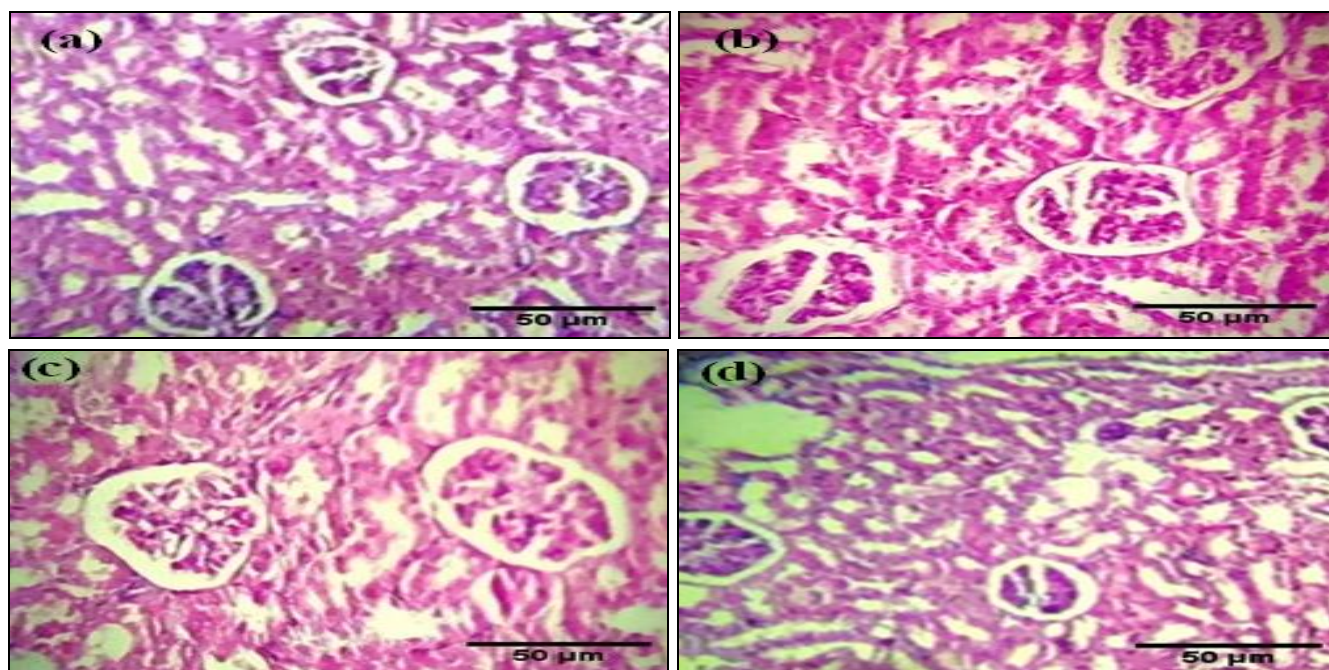


FIG. 1: HISTOLOGICAL EXAMINATION OF KIDNEY TISSUE; (A): DISTILLED WATER GROUP: NORMAL TISSUE×100; (B): SYZYGIUM AROMATICUM GROUP: NORMAL TISSUE×100; LAURUS NOBILIS GROUP: NORMAL TISSUE×100; (D): FUROSEMIDE GROUP: NORMAL TISSUE×100

DISCUSSION: The diuretic effect of *Syzygium aromaticum* and *Laurus nobilis* was evaluated in normal rats and compared with distilled water (negative control) and furosemide (positive control); the results showed that oral administration of the aqueous extract of *Syzygium aromaticum* and aqueous extract of *Laurus nobilis* induced an increase in urine volume and urinary excretion of sodium, and chloride, no effect was shown in serum electrolytes by both plants, while furosemide causes a decrease in serum potassium. Furosemide is a loop diuretic that increases urine flow via the inhibition of co-transport of $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$, at the ascending part of the loop of hense, it causes urinary excretion of sodium, potassium, and chloride¹⁴.

Furthermore, the results showed that the clearance of creatinine was increased by furosemide and by both plant extracts, this result could be explored to increase creatinine clearance in renal failure¹⁵. Concerning carbonic anhydrase inhibition, according to vogel 2002, Carbonic anhydrase inhibition can beleft out at ratios between 1.0 and 0.8, and we can assume a slight to strong anhydrase carbonic inhibition with decreasing ratios¹⁶. This indicated that furosemide had a slight effect on

anhydrase carbonic inhibition while no effect was shown by *Syzygium aromaticum* extract and *Laurus nobilis* extract, in addition, the ratio Na^+ / K^+ does not change with both plant extracts. These features suggest that the aqueous extracts of *Syzygium aromaticum* and *Laurus nobilis* might have a mechanism of action similar to thiazide diuretics, which increase the urinary excretion of sodium and chloride by blocking the co-transport $\text{Na}^+ / \text{Cl}^-$ at the distal tubule¹⁴.

Thiazide diuretics, as well as loop diuretics, are known by hypokalemia,¹⁷ while no significant change showed in the serum potassium levels by the peros administration of *Syzygium aromaticum* extract or *Laurus nobilis* extract. A possible explanation for this might be the richness of these plants in potassium^{18,19}. Another important finding was that no pathological change in the kidney tissue was caused by aqueous extracts of *Syzygium aromaticum* or *Laurus nobilis* **Fig. 1**.

The phytochemical analysis of *Syzygium aromaticum* and *Laurus nobilis* showed the richness of these plants in flavonoids compounds such as quercetin, kaempferol, rutin, catechin, luteolin, and apigenin²⁰⁻²².

Several studies thus far have linked flavonoids with diuretics effects; for example a study revealed that luteolin has a diuretic and natriuretic effect²³, Junior *et al.*, showed that isoquercitrin is responsible for a diuretic and potassium-sparing effect²⁴ and Mariano et al showed the diuretic and saluretic effects of (-)-epicatechin²⁵. Therefore, it seems possible that the diuretic effect of *Syzygium aromaticum* extract and *Laurus nobilis* extract could be attributed to their flavonoids content.

The diuretic characteristic of *Syzygium aromaticum* and *Laurus nobilis* can be explored to investigate other pharmacological properties such as hypotensive effect²⁶.

CONCLUSION: The most interesting finding in the current study was that daily oral administration of aqueous extracts of *Syzygium aromaticum* or *Laurus nobilis* increases diuresis in normal rats, over a period of nine days, with no significant alterations in blood electrolytes and kidney tissues. Further, studies are needed to clarify the mechanism of action and the active ingredient responsible for the diuretic effect.

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CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

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